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PLFA Profiling of Microbial Community Structure Influencing Ecosystem Restoration in Chronosequence Iron Mine Overburden Spoil

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Abstract: Phospholipid fatty acid analysis (culture-independent approach) provides a set of molecular markers for microbial taxa and consistently used to discriminate microbial communities of different origin and land uses. PLFA profiling provides an approach for microbial community assessment and their changes regulating ecosystem restoration. PLFAs are synthesized during microbial growth, rapidly degraded following cell death and hence can reliably reflect living microbial communities involved in ecosystem structure and function. Relative distribution of 75 PLFAs across the sites revealed significant differences in microbial community structure in seven different iron mine overburden spoil with variation in Shannon diversity index from 2.4905 (IB_4) to 2.8490 (IB_2) and Pielous evenness index from 0.7005 (IB_0) to 0.7556 (IB_4). The ratio of gram-positive to gram-negative exhibited a decline trend from IB_0 (2.129) to IB_{25} (1.137) over time. Fungal to bacterial ratio exhibited an increasing trend from IB_0 (0.0233) to IB_{25} (0.0640) and was found to be higher in NF soil (0.1116), revealing the sign of ecosystem restoration over time. F:B ratio showed positive correlation with pH ($r = 0.872$, $p<0.001$), moisture ($r = 0.888$, $p<0.001$). Based on the relative distribution of PLFAs, the principal component analysis and cluster analysis can able to discriminate different mine overburdens into independent clusters. Redundancy analysis can contribute for soil quality assessment based on the shift in 75 PLFAs. Thus, PLFA profiling evaluated the broad scale patterns of distribution of microbial community structure influencing soil quality and can be used for monitoring the restoration of iron mine spoil over time.

Keywords: Iron mine spoil; microbial community structure; PLFA; reclamation.

I. INTRODUCTION

Soil microbial community structure is being marked as ecologically relevant endpoint and realistically incorporated for assessment of potential risks associated with anthropogenic disturbances, which influence microbial community composition at multiple spatial extent and are linked closely to soil environmental heterogeneity [1]. The shift in microbial community structure and composition is regarded as sensitive indicator of microbial activity reflecting soil quality [2]. Microbial community structure is also considered as an inherent factor in determining the biogeochemical cycles and organic matter turnover in soil [3]. Besides, the assessment of microbial community composition is pre-requisite to determine biodiversity, ecological structure and sustainability [4]. Microbial community dynamics provides information about the soil quality status. Soil exhibiting higher microbial diversity is the characteristic feature of fertile soil subsystem where the microbes are the key players involved in nutrient turnover [5]. In contrast, the degraded soil with low microbial diversity is often hardly responds to environmental changes [6]. Therefore, it is essential to quantify the relationship between microbial community structure and ecosystem function.

An approach to detect possible changes in soil microbial community can be analyzed through PLFA profiling [7]. PLFA analysis is culture-independent approach in order to determine the variation in microbial community composition and used as an approximation for taxonomic diversity assessment [7-11]. PLFA analysis has been applied to elucidate different strategies employed by soil microorganisms to adapt the changed environment along fertility gradient [12], management practices under wide range of perturbations and alliance with changes in vegetation during succession [13-14]. Different subsets of microbes have unique PLFA patterns, which reflect microbial community fingerprint to determine the characteristic features of microbial community directly in natural habitat [14]. Community-level PLFA profiles have been found to be useful in detecting the responses of soil microbial communities with respect to the variation in land use patterns or disturbances [15-17]. PLFA analysis can reliably reflect the biomass of microbial community as it is exclusively found in cell membrane of microbes. However, the detection and interpretation of decline in PLFA abundance is difficult

since it assumes the degradation of their phospholipids rapidly to diglycerides following cell death [18-19]. Therefore, the PLFA content is used as an index of viable microbial biomass [20-22]. Besides, the analysis of microbial populations by PLFA profiling provides direct information about their identification, classification and microbial community composition, which overcomes the problems associated with the culture techniques [23]. PLFA profile does not provide actual species composition but instead reflects the overall microbial community structure [24].

Phospholipid composed of single molecule of glycerol with two OH groups being replaced by two fatty acids by ester or ether linked showing the properties of hydrophobic tail, whereas third OH group is linked with phosphate group representing hydrophilic head. Microbial fatty acids are C_{12} to C_{24} long but the membrane fatty acids are usually C_{14} to C_{20} long [22]. PLFA can be grouped into ester-linked phospholipid (EL-PLFAs, 60-90%) and non-ester linked phospholipid (NEL-PLFAs, 10-40%) fatty acids. EL-PLFAs are subdivided into ester-linked unsubstituted (EL-UNFAs) and hydroxyl substituted (EL-HYFAs) fatty acids. EL-UNFA includes saturated (EL-SATFA), monounsaturated (EL-MUFA) and polyunsaturated (EL-PUFA) fatty acids. EL-SATFA has two sub-groups: branched chain (BRANCs) and straight chain (STRAs) fatty acids. NEL-PLFAs include unsubstituted (NEL-UNFA) and hydroxyl substituted (NEL-HYFA) fatty acids [13]. Phospholipids can exist with varying chain length, saturation and branching [25]. Even though phospholipids occur in different life forms, the fatty acid side chains between life forms are quite unique. Polyunsaturated fatty acids (18:3ω3c) are found in plants and cyanobacteria but are not present in bacteria. Monounsaturated (ω-7), odd-chain saturated (15:0), branched-chain (iso/anetiso and 10-methyl) and cyclopropane (19:0 cyclo ω7c) fatty acids are mostly synthesized by bacteria [25] and therefore used for microbial community fingerprint [26-28]. The fatty acid extracted from sediments can able to classify distinct microbial groups such as microeukaryotes (PUFA), aerobic prokaryotes (MUFA), gram-positive and anaerobic bacteria (saturated and branched fatty acids; C_{14}-C_{16}), gram-positive bacteria (branched-chain fatty acids; iso/anteiso). Besides, LPS-OH fatty acids were used as reliable indicators of gram-negative bacteria because of their existence in lipid portion of lipopolysaccharides in cell wall [29].

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 and 24:0) was considered to be predominantly of bacterial origin [7]. PLFAs (16:1ω7c, cy17:0, 18:1ω7c and cy19:0) are the representatives of heterogeneous groups of soil microbes most prevalent in gram-negative bacteria [30-32], the iso and antesio branched PLFAs (a13:0, a15:0, i15:1ω6c, a15:1ω9c, a16:0, a17:0, a17:1ω7c and i17:1ω9c) typically represents gram-positive bacteria [31-32]. Besides, sulfate-reducing bacteria including other anaerobic bacteria were represented by saturated and branched (C_{16}-C_{19}) fatty acids [33]. PLFAs (18:1ω9c, 18:2ω6c and 18:3ω6c) are used to represent common fungi [31-32, 34-35]. The unsatured fungal biomarker 16:1ω5c is typical for arbuscular mycorrhizal fungi [32]. Methyl branched PLFAs 10Me16:0, 10Me17:0, 10Me17:1ω7c, 10Me18:0, 10Me19:1ω7c and 10Me20:0 representing actinomycetes [36-37], PLFAs 14:1ω7cDMA, i15:0DMA, 16:1ω7cDMA, 18:0DMA, 18:2DMA, 19:0cy for anaerobes [13, 38], PLFAs 16:1ω7c, 18:1ω7c for aerobic bacteria [39], PLFAs 16:1ω7c and 16:1ω8c for methanobacter [37, 40], PLFAs i17:1ω7c, 11:1ω6c, 10Me16:0 [41] and PLFA 10Me18:0 for sulfate reducing bacteria [13, 42].

PLFA profiling is rapid and reliable way of assaying the biomass and composition of microbial communities [43-44]. Besides, it is considered as more sensitive and reproducible technique to quantify the shift in microbial community composition and soil nutritional/physiological status compared to culture dependent methods [4]. The shift in PLFAs can reflect overall changes in microbial community structure, which can be used as indicators of disturbances and provided valuable information regarding the implementation of reclamation strategies [45-48]. Besides, PLFAs represent set of molecular markers for different microbial taxonomic groups [27, 49-50]. Thus, PLFA profiling provides an accurate census of the current living communities in the ecosystem [50-51]. Keeping in view, the present investigation was designed to provide comparative assessment of microbial community structure in seven iron mine overburden spoil in chronosequence over time and the nearby forest soil. Further, the fungal to bacterial PLFAs and gram-positive to gram-negative PLFAs ratio within the soil microbial communities were estimated not only to determine the reclamation progress but also to understand the relationship between microbial community structure and ecosystem function.

II. MATERIALS AND METHODS

A. Study site

The present study was carried out in iron mining area located at Noamundi in the revenue district of West Singhbhum, Jharkhand, India (85° 28' 02.61" east longitude and 22° 8' 33.93" north latitude) (Figure 1). The study site is situated away from the mean sea level *i.e.* about 581 m altitude with the mean annual temperature 19.67°C and humidity 20%. Tropical dry deciduous forest is considered to be natural vegetation of the area. However, extensive iron mining activities led to decline of forest cover and generated a number of

abandoned iron mine overburdens. In the present study, seven iron mine overburdens in chronosequence have been selected based on the time elapsed since inception such as fresh iron mine spoil (IB_0), 2yr (IB_2), 4yr (IB_4), 6yr (IB_6), 8yr (IB_8), 15yr (IB_{15}), and 25yr (IB_{25}). Besides, the nearby native forest soil (NF) adjacent to core mining area was selected for comparison.

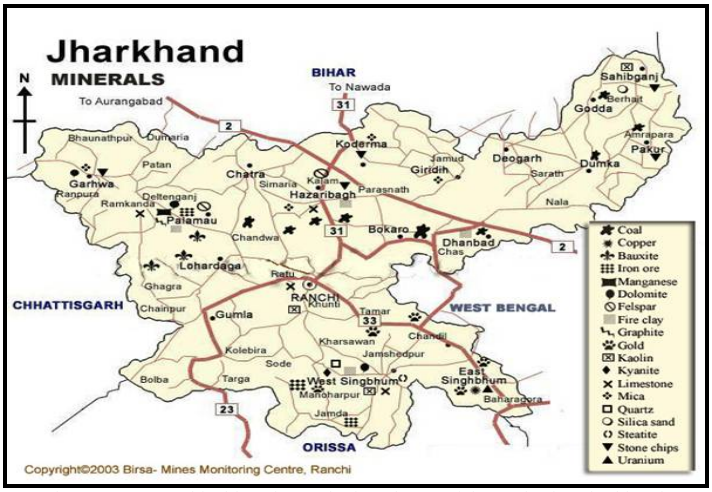


Figure 1. Geographical location and mineral map of the study site, Jharkhand.

B. *Mine spoil sampling*

Spoil sampling was done in accordance with the microbiological method from seven different age series iron mine overburdens (IB_0, IB_2, IB_4, IB_6, IB_8, IB_{15} and IB_{25}) and NF soil [52]. Each site was divided into 3 blocks and five mine spoil samples were collected randomly from 0-15 cm soil depth by digging pits of 15 cm^3 size, which is referred as 'sub-samples' and mixed thoroughly to form 'composite sample'. Similar sampling strategies have been followed to obtain three composite samples from different age series iron mine overburden. The samples were subjected to sieving (0.2 mm mesh) and stored at 4°C for further analysis.

C. *Phospholipid fatty acid (PLFA) analysis*

Lipids extraction based on fractionation and quantification was performed [53], which is simple, fast and used for microbial community analysis including non-culturable microbes. Lyophilized soil sample (5g dry weight) was sonicated with phosphate buffer, methanol and chloroform (4:10:5 v/v/v) for 10 min in sonicating water bath at room temperature and rotated end over end for 2hr. The mixture was centrifuged at 2500 rpm for 10 min and liquid phase was transferred followed by addition of equal volume of distilled water and chloroform (5:5 v/v), shaken vigorously and incubated for 24hr for separation of phases. The bottom organic phase was evaporated under nitrogen (N_2) and stored at -20^{0}C. Lipid fractions were separated by solid phase extraction (SPE) chromatography by washing the silica gel column with chloroform. After loading the extract in chloroform the neutral, glyco and phospholipids were eluted with chloroform, acetone and methanol respectively followed by the evaporation of the phospholipids fraction under N_2 and stored at -20^{0}C. The trans-esterification of fatty acids was performed with equal volume of methanol and toluene (1:1 v/v) following alkaline methanolysis of phospholipids by methanolic KOH at 37^{0}C for 15min. The resulting ester-linked fatty acid methyl esters (FAME) was dissolved in isooctane or hexane, acetic acid and double distilled water mixture (2:0.3:2 v/v/v) and vortexed. The top (organic) phase was removed following the extraction process repeatedly with addition of hexane and the combined phase was evaporated under N_2 and stored at -20^{0}C. The extracts were cleaned using NH_2 SPE column and the samples were dissolved in equal volume of hexane: methyl tert-butyl ether (1:1 v/v) and quantified through GC-MS.

PLFA nomenclature follows the common convention of A:BωC [54], where the total number of C atoms in the fatty acids is denoted as 'A' and the number of double bonds as 'B'. The position of the double bond is defined by a symbol 'ω' followed by the number of carbons 'C' from the methyl end of fatty acid molecule. The prefixes *cis* and *trans* configuration are indicated by c and t, i and a refer to *iso* and *anteiso* branching, *br* indicates an unknown methyl branch position, *cy* refers to cyclopropyl fatty acids. Hydroxyl groups are indicated by 'OH'. The 10Me indicates the presence of methyl group in C_{10} from the carboxyl end of the fatty acid [55-56].

D. *Statistical analysis*

PLFA profiles were analyzed using Sherlock PLFA tool (Version 1.1). Shannon's diversity index or Shannon-Weaver index (H) was calculated as ($-\Sigma p_i \ln p_i$), where p_i is the peak area of i^{th} peak over the area of all peaks. Pielou's evenness index (J) was calculated as ($H/Hmax$), where H is the no. derived from Shannon diversity index and $Hmax$ is the maximum value of H ($Hmax = \ln R$; R denotes PLFA richness). Principal component analysis was performed in order to discriminate seven iron mine overburdens based on the relative distribution PLFAs across the sites using SPSS (Version 18.0). Besides, cluster analysis using distance matrix based on the relative distribution of 75 PLFAs across the sites was performed to illustrate the relatedness among them. RDA was performed using XLSTAT-2014 (Version 2.03).

III. RESULTS AND DISCUSSION

Community level PLFA profiling has been used to determine the qualitative as well as quantitative changes in soil, which provides broad diversity measure of microbial community composition associated with their activities and the nature of interaction among them in terrestrial ecosystems [4, 16, 17, 19, 57]. Besides, the existence of different functional groups responds differently to prevailing environmental conditions in different ecosystems, which affects the microbial community composition. Certain PLFAs with specific functional groups are used as potential biomarkers for fingerprinting the existence of microbial communities based on the relative abundance of soil microorganisms [31-32, 37, 42, 50, 58-59].

A. PLFA profiles of mine spoil

The relative contribution of 75 PLFAs representing microbial community structure across seven different age series iron mine overburden spoil in chronosequence and nearby NF soil revealed marked variation (Table 1). It is evident that the PLFA profiles were dominated by saturated fatty acids (14:0, 15:0, 16:0, 17:0 and 18:0), branched saturated fatty acids (i15:0, a15:0, i16:0 and i17:0), cyclopropyl fatty acids (cy17:0 and cy19:0), MUFA (16:1ω9c, 18:1ω9c and 18:1ω9t), PUFA (18:2ω6), hydroxyl fatty acid (3OH14:0) (Table 1). Higher relative abundance of three fungal PLFAs [18:1ω9c (oleic acid), 18:2ω6c (linoleic acid), 18:3ω6c (gammalinoleic acid)], which accounted for 4.19%, 1.98% and 0.97% respectively were observed in NF soil [31-32, 35, 37, 60]. PLFA 18:1ω9c is reported to be most common in fungal species [13]. High prevalence of fungal PLFA in NF soil may be attributed to the availability of higher amount of recalcitrant polymeric phenolic compounds such as lignin and tannin and principally responsible for lignin degradation [61]. Similarly, higher relative abundance of arbuscular mycorrhizal fungal PLFA 16:1ω5c (cis-11-palmitoleic acid) were estimated in NF soil (6.15%) compared to different age series iron mine overburden spoil [32]. PLFA 16:1ω5c derived from arbuscular mycorrhizal fungi is known to contribute substantially to fungal biomass in NF soil [60], which is influenced by the changes occur in soil organic C [51, 62]. The methyl branched PLFAs representing actinomycetes (10Me16:0, 10Me22:0, 10Me18:0 and 10Me17:1ω7c) were not detected in NF soil (Table 1) [13, 36-37]. Higher relative abundance of methanobacter PLFA 16:1ω7c was exhibited by NF soil compared to different iron mine overburden spoil [37].

Table 1. Percentage composition of 75 PLFAs in the seven different age series iron mine overburden spoil as well as nearby NF soil.

PLFAs	IB_0	IB_2	IB_4	IB6	OB_8	OB_{15}	OB_{25}	NF
10:00	0.17	0.29	0	0	0	0	0	0
10:0 3OH	0	0.31	0	0	0	0	0	0
11:00	0	0	0.71	0.56	0	0	0	0
a11:0	0	0	3.1	2.45	0	0	0	0.7
i11:0	0	0	0	1.15	1.22	0	0	0
12:00	26.7	0	27.25	26.68	24.12	25.62	29.58	13.08
a13:0	2.21	2.46	2.99	1.57	1.69	1.8	2.56	3.36
13:0 DMA	0	0.13	0	0	0	0	0	0
13:1 w4c	0.21	0.3	0.49	0.38	0	0.3	0.3	1.27
13:1 w5c	0	0	0	0	0.21	0	0	0
14:00	0.95	1.03	1.26	1.13	0.85	2.48	1.12	1.67
i14:0	0.7	0	0	0	0	0	0	0.31
a14:0	0.19	0	0	0.11	0.13	0.13	0.29	0.23
14:0 DMA	0.26	0	0	0.14	0.21	0	0.19	0
14:1w8c	0.31	0.28	0	0.25	0.27	0.2	0.23	0.48
14:1 w5c	0.39	0.63	0	0.25	0.39	0.22	0.28	0.24
15:00	0.18	0.22	0	0.21	0.21	0.36	0.21	0.97
a15:0	3.13	3.66	4.8	2.57	2.73	3.04	3.84	4.26
15:0 DMA	0	0	0	0	0.23	0	0	0
i15:0 DMA	0.2	0.37	0	0.25	0.19	0.16	0.2	0.31
i15:1 w9c	0.38	0	0	0.23	0.33	0	0.35	0
a15:1 w9c	0.37	0.41	0	0.27	0.33	0.31	0.31	0
15:1 w8c	0.62	0.52	0.48	0.31	0.36	0.42	0.48	0
15:1 w9c	0	0.26	0	0.22	0.19	0.14	0.15	0

15:3 w3c	0	21.54	0	0	0	0	0	0
16:00	10.44	14.28	4.53	11.15	13.52	17.13	9.94	5.25
i16:0	0	0	0	0	0.13	0.32	0.3	0.54
16:0 2OH	0	0	0.77	0	0	0	0	0
16:0 N alcohol	1.06	10.07	0.83	0.95	1.22	0.92	0.92	1.41
10 Me16:0	0.51	0.69	0	0	0	0.66	0	0
16:1 w5c	0	0.41	0	0	0	0.35	0.7	6.15
16:1 w7c	0	1.23	0	0	0	0.67	0.81	1.25
16:1 w7c DMA	0	0	0	0	5.88	0	4.82	7.67
16:1 w9c DMA	5.68	7.32	1.64	0.95	0	0	0	0
16:2 DMA	2.04	2.36	3	2.95	2.91	2.36	2.49	2.54
17:00	0	0	0	0	0.2	0.96	0.42	0.49
a17:0	2.24	0	3.81	3.64	2.1	2.45	2.93	2.84
17:0 cy w7c	0	0.77	0	0	0	0	0	0
17:1 w5c	0.25	0	0.43	0.32	0.29	0.35	0.32	0
a17:1 w7c	0.42	0.68	0.66	0.37	0.48	0.46	0.52	0.61
i17:1 w9c	1.73	3.98	0.47	0.54	0.76	0.34	0.45	0.75
10 Me 17:1 w7c	0	0.17	0	0	0	0	0	0
17:1 w8c	0	0.6	0	0	0	0.66	0	0
18:00	5.97	7.46	2.65	6.21	7.9	10.94	4.67	5.93
18:0 DMA	0.15	0.24	0	0	0	0	0.16	0
10 Me 18:0	0	0	0	0	0	0.15	0.15	0
18:1 w5c	0.28	0	0	0	0.22	1.28	0.75	1.62
18:1 w7c	0.62	1.44	0	0.55	0.6	0.73	0.96	1.01
18:1 w9c	2.64	3.62	2.67	3.14	3.11	5.96	3.25	4.19
18:2 DMA	0.2	0.24	0	0	0.18	0.14	0	0
18:2 w6c	0.63	0.91	0.43	0.78	0.78	1.03	0.88	1.98
18:3 w6c	0.83	1.02	0.97	0.86	1.34	1.14	0.8	0.97
19:0 cy w7c	0.2	0.45	0	0.21	0.23	0.27	0.36	0.52
19:0 cy w9c	0	0	0	0	0	0.12	0	0
19:1 w8c	0.18	0	0.43	0.31	0.18	0.28	0.29	0.31
19:3 w3c	11.25	0	20.54	11.08	10.14	10.89	15.12	10.22
19:3 w6c	0.42	0	0	0	0.39	0	0	0
20:00	0.26	0.86	3.15	1.98	0.25	0.76	0.66	0.86
20:1 w4c	0.27	0.38	0.57	0.33	0.27	0.33	0.5	0.61
20:1 w9c	0.16	0.27	0	0.21	0.15	0	0.39	0.41
20:3 w6c	0.25	0	0	0	0	0.24	0	0
21:00	0	0	0	0	0	0.12	0	0
21:1 w3c	0	0	0	0	0	0	0.39	0
21:1 w5c	0	0	0	0	0	0.19	0	0
21:1 w8c	0.21	0.38	0.51	0.42	0.23	0.15	0.41	0.45
21:3 w3c	7.95	0	0	7.34	7.18	0	0	8.05
22:00	0.32	0.21	0	0.23	0.28	0.94	1.13	1.27
i22:0	0	0	0	0	0	0.7	0.39	0.42
10 Me 22:0	0.22	0.26	0	0.24	0.19	0	1.3	0
22:1 w9c	0.34	0	0	0	0.33	0	0	0.24
23:00	0	0	0	0	0	0.15	0	0
23:3 w3c	4.89	6.68	10.85	6.33	4.33	0	0	3.04
23:4 w6c	0.28	0.38	0	0	0.39	0	0	0.43
24:00	0.16	0.22	0	0.18	0.18	0.68	1.08	1.09
24:3 w3c	0	0	0	0	0	0	1.65	0

B. Distribution of PLFAs

PLFA pattern is used to describe the microbial community composition in different age series iron mine overburden spoil in chronosequence as well as nearby NF soil, grouped into distinct categories such as microeukaryotes (PUFA), aerobic prokaryotes (MUFA), gram-positive and other anaerobic bacteria (saturated and branched fatty acids ranges from C_{14} to C_{16}), anaerobic bacteria (saturated and branched fatty acids ranges from C_{16} to C_{19}) (Table 2) [4, 33]. The PUFAs are considered as the signature fatty acids for eukaryotes, which range from 21.55% (IB_{25}) to 33.61% (IB_4). MUFAs representing aerobic prokaryotes can occur both in gram-negative and gram-positive bacteria that ranges from 10.12% (IB_8) to 15.53% (IB_2). However, their relative contribution to total PLFA in gram-positive bacteria was found to be minimal (20%) and hence MUFAs can be considered as biomarkers for gram-negative bacteria [63]. Relatively higher level of unsaturated fatty acids with low level of PUFAs supported the bacterial dominance. Relatively higher level of straight chain PLFAs was exhibited by IB_{25} (44.91%) compared to different age series iron mine spoils. Branched chain PLFAs varies from 9.31% (IB_8) to 14.53% (IB_4). Branched chain fatty acids have been used as biomarkers for bacteria including anaerobic and sulfate-reducing bacteria. Branched chain fatty acids (iso/anteiso) are characteristic features of gram-positive bacteria, whereas cyclopropyl fatty acids are common in some gram-negative and anaerobic gram-positive bacteria [63]. The differences in relative distribution of

branched and MUFAs are used as useful markers for the proportion of gram-positive and gram-negative bacteria [33, 57].

Table 2. Distribution of different PLFAs groups (%) in the different age series iron mine overburden spoil as well as nearby NF soil.

Sample	Straight	Branched	Hydroxy	MUFA	PUFA	DMA	18:1 w9c	18:2w6	10- methyl
IB_0	40.21	10.91	0.15	12.03	31.01	2.99	1.34	0.41	0.73
IB_2	31.65	11.72	0.31	15.53	33.12	3.16	1.62	0.65	1.12
IB_4	33.69	14.53	0.77	10.67	33.61	3.31	2.67	0.63	0
IB_6	38.94	10.15	nd	10.54	32.48	3.52	3.14	0.78	0.24
IB_8	43.53	9.31	nd	10.12	28.42	3.87	3.42	0.91	0.19
IB_{10}	43.67	11.52	nd	10.61	24.94	3.47	3.96	1.13	0.31
IB_{25}	44.91	11.83	nd	12.73	21.55	3.23	3.99	1.25	0.15
NF	45.12	11.95	nd	12.85	20.32	2.75	4.19	1.98	0.32

nd: beyond detectable limit.

Gram-negative bacteria contain unique hydroxyl fatty acids in lipopolysaccharides as cell wall composition, which act as an indicator of gram-negative bacteria in environmental samples [29, 64]. It is evident from the study that soil microbes with hydroxyl fatty acids were confined to IB_0 (0.15%), IB_2 (0.31%) and IB_4 (0.77%). Higher relative abundance of methyl branching PLFAs was observed in IB_2 (1.12%) compared to different age series iron mine spoil (Table 2). The distribution of MUFAs and PUFAs in NF soil accounted to 12.85% and 20.32% respectively (Table 2). Relatively higher level of PLFAs 18:1w9c (4.19%) and 18:2w6c (1.98%) representing fungi were observed in NF soil compared to different iron mine spoil. The study indicated that the differences in PLFA profiles could be attributed to the variation in lipid contributing microbial communities; environmental conditions and microbial amelioration during spontaneous succession in iron mine overburden spoil over time [65-66].

C. Microbial community composition

The short-term responses of microbial mediated processes and community structure to perturbation constitute important aspects of soil quality assessment and productivity. Thus, it is necessary to analyze their relative distribution or composition and microbial diversity, which provide better understanding of soil quality. Such analysis of microbial community composition has relied on the relative distribution of microbial PLFAs. Besides, PLFAs have several features that reinforce their use as indicator of environmental stress. They respond to environmental disturbances either by phenotypic plasticity or altering PLFAs composition in microbial membrane and thereby shifting microbial community structure [50]. Marked differences in microbial community composition were observed across the different age series iron mine overburden spoil over time (Table 3).

Table 3. Relative distribution of microbial communities (%) in different age series iron mine overburden spoil as well as nearby NF soil.

Soil sample	Gram positive	Gram negative	Anaerobes	Actinomycetes	A.M. Fungi	Fungi	Methanobacter	Eukaryote
IB_0	17.89	26.14	5.06	0.91	0	0.54	0.65	48.81
IB_2	15.32	23.52	5.31	1.37	0.62	0.72	0.98	52.16
IB_4	14.35	21.91	4.83	0	0	1.07	0	57.84
IB_6	14.08	19.75	5.24	0.41	0	1.24	0	59.28
IB_8	12.36	17.64	6.92	0.37	0	1.52	0	61.19
IB_{10}	11.18	14.48	6.77	1.02	0.85	2.13	0	63.57
IB_{25}	10.71	13.78	6.09	0.33	1.33	1.83	0	65.93
NF	10.12	13.25	5.06	0.45	2.13	2.74	0	66.25

nd: beyond detectable limit.

The fresh iron mine spoil represents disequilibriated geomorphic system with altered physicochemical properties, which disrupts soil quality/stability and pedogenic processes [67]. Relatively higher level of MUFAs [65, 68] with lower level of PUFAs were reported as biomarkers for gram-negative bacteria [63], which explained relative abundance of gram-positive bacteria in IB_0 (17.89%) (Table 3). The existence of hydroxyl PLFAs substantiated the higher occurrence of gram-negative bacteria in IB_0 (26.14%) compared to different age series iron mine spoils and NF soil [29, 64]. Further, higher level of gram-positive bacteria (17.89%) was estimated in IB_0, which may be due to higher relative occurrence of branched chain fatty acids [68-69]. The study revealed higher relative dominance of gram-negative bacterial PLFAs in heavy metal contaminated mine spoil (IB_0) with concomitant decrease in gram-positive bacterial PLFAs in chronosequence iron mine spoil over time [9, 13, 70]. Higher level of DMA PLFAs indicates relatively higher distribution of anaerobes in IB_8 (6.92%) compared to different age series iron mine spoil [13, 38, 42]. The methyl-branched PLFAs represent the dominance of actinomycetes in IB_2 (1.37%) [13, 36, 37], which may be due to their potentiality to withstand

water stress by resisting plasmolysis and maintain 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 pore spaces during soil desiccation [71]. The occurrence of relatively lower fungal PLFAs (18:1ω9c, 18:2ω6, 18:2ω9c) indicated minimal fungal abundance in IB_0 (0.54%). The methanobacter population was confined to IB_0 (0.65%) and IB_2 (0.98%). Minimal existence of longer chain fatty acids in IB_0 (48.81%) indicated comparatively lower input from microeukaryotes, which may be due to the interaction of heavy metals with microbial membrane proteins resulting disturbances in protein conformations and activities [9, 70, 72].

The ability to maintain microbial community composition, nutrient turnover and functioning after disturbance defines the resistance capacity of a soil subsystem. Resilience expresses the degree of response of the system impacted by disturbances and the rate of recovery in the original versus restored state of system. In addition to abiotic factors, soil microbial community composition is considered as major components of soil resilience due to their key role in nutrient cycling. Therefore, the microbial community composition in different age series iron mine overburden spoil in chronosequence over time should be compared with IB_0. Comparatively higher level of MUFAs and PUFAs were detected in IB_2 compared to IB_0, which explained higher occurrence of gram-negative bacteria in IB_2 (23.52%) [65, 68]. Besides, the relative dominance of hydroxyl PLFAs in IB_4 revealed the occurrence of gram-negative bacteria in IB_4 compared to different iron mine spoils (Table 3) [29, 64]. Besides, higher level of gram-positive bacteria in IB_2 (15.32%) may be due to higher occurrence of branched chain fatty acids. Relatively lower level of anaerobes in IB_2 (5.31%) was attributed to lower occurrence of DMA PLFAs (Table 3). Higher level of fungal PLFAs (18:1ω9c) revealed higher fungal dominance in IB_2 (0.72%) compared to IB_0 due to gradual establishment of vegetation and inputs of allochthonous material [73-74]. Methyl-branched PLFAs representing actinomycetes was found to be relatively higher in IB_2 (1.37%) compared to IB_0 (0.91%) [13, 36-37]. Distribution of methanobacter was confined to IB_2 (0.98%) and IB_0 (0.65%) due to the presence of 10-methyl branched fatty acids. Further, higher occurrence of long chain fatty acids and PUFAs supported higher relative distribution of microeukaryotes in IB_2 (52.16%) compared to IB_0. Thus, the recovery of resource heterogeneity and pool size following restoration progress would indicate resilience of the system leading to variation in soil microbial community composition.

PLFA profiles suggested higher level of gram-negative bacteria in IB_4 (21.91%) and IB_6 (19.75%) compared to IB_8 (17.64%), which may be due to the higher level of MUFAs in IB_4 and IB_6 [63, 65, 68]. Higher relative distribution of gram-negative bacteria was observed in IB_{15} (14.48%) compared to IB_{25} (13.78%). Higher distribution of gram-positive bacteria was observed in IB_4 (14.35%) compared to IB_6, IB_8, IB_{15} and IB_{25}, which may be due to higher occurrence of branched chain fatty acids in IB_4 (43.53%). Lower level of anaerobes was estimated in IB_4 (4.83%) compared to IB_6 (5.24%), which may be due to lower occurrence of DMA PLFAs. The distribution of actinomycetes was not observed in IB_4 due to absence of methyl-branched PLFAs. Methyl-branched PLFAs was found to be higher in IB_{15} (1.02%) compared to IB_{25} (0.33%). Higher level of fungal PLFAs (18:1ω9c) revealed higher fungal dominance in IB_{15} (2.15%) compared to different age series mine spoils. Higher relative distribution of PLFA 16:1ω5c reflects the dominance of arbuscular mycorrhizal fungi in IB_{25} (1.33%) compared to IB_{15} (0.85%) and IB_2 (0.62%). However, the distribution of arbuscular mycorrhizal fungi was not observed in IB_0, IB_4, IB_6 and IB_8. Higher longer chain fatty acids indicate relatively higher inputs from microeukaryotes in IB_{25} (65.93%) compared to different iron mine spoils.

The distribution of gram-positive, gram-negative, anaerobes and actinomycetes in NF soil was found to be 10.12%, 13.25%, 5.06% and 0.45% respectively (Table 3). Relative abundance of gram-negative as compared to gram-positive bacteria in NF soil indicated profound effect of vegetation on mine spoil genesis and lipid profile. Higher relative distribution of arbuscular mycorrhizal fungi (2.13%), fungi (2.74%) and microeukaryotes (66.25%) were observed in NF soil, which may be due to allochthonous inputs, root turnover and symbiotic nitrogen fixation contributed to formation of highly localized soil resources characterized by higher level of organic C and N that are believed to support more diverse heterotrophic microbial population. Greater PLFA diversity also concurred with studies on vegetation succession [75-76]. Further, fungi are adapted to degrade lignin and formation of organic matter [61]. Comparative analysis based on the distribution of PLFAs suggested that the heavy metal contamination in mine spoil resulted decline in PLFAs (a15:0, 16:1ω5c, 18:1ω7c, 18:1ω9c, 18:2ω6c and 18:3ω6c) in IB_0 compared to NF soil [9, 13, 24, 69, 72].

Further, PLFA markers were used to quantify the relative abundance of gram-positive (i14:0, i15:0, a15:0, i16:0, 10Me16:0, i17:0, a17:0, 10Me17:0) to gram-negative bacteria (15:1ω4c, 16:1ω7c, 16:1ω9c, cy17:0, 17:1ω9c, 18:1ω7c, 18:1ω9c, cy19:0; cy19:0ω7c) ratio in different age series iron mine overburden spoil and NF soil [27, 42, 62, 77]. The ratio of gram-positive to gram-negative bacteria exhibited a decline trend from IB_0 (2.129) to IB_{25} (1.137) and was found to be minimum in NF soil (1.104) compared to different iron mine overburden spoil (Figure 2). Such increase in gram-negative bacteria may be attributed to gradual improvement in organic C supported by vegetation development in chronosequence iron mine overburden spoil over time [67], which provides more stable and readily available substrate for supporting higher microbial activity of gram-negative bacteria [78]. Several investigations have suggested that the gram-negative bacteria were closely associated with MUFAs, which correspond to the gradual increase in organic matter and high substrate

availability [58, 79]. Thus, the combined effect contributed by the changes in aboveground and belowground inputs would influence the microbial community structure by altering C inputs from root exudates and litter in chronosequence iron mine overburden spoil influencing the pace and progress of mine spoil restoration over time [19, 35, 66].

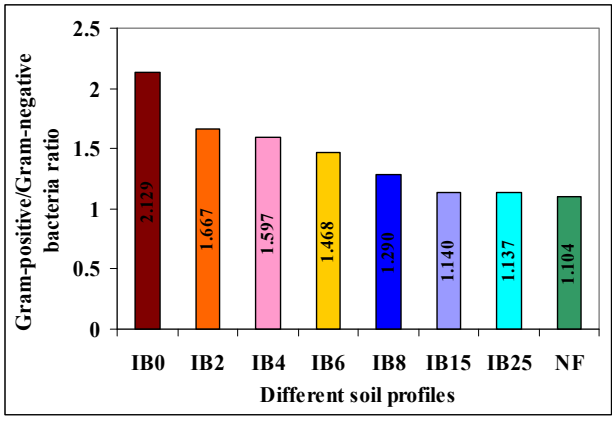


Figure 2. Gram-positive to gram-negative bacteria ratio in the different age series iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) as well as nearby NF soil across the sites.

D. Shannon-weaver diversity index

The numerical strength and biomass of microorganisms affect ecosystem functioning. Microorganisms can change, modify and regulate microenvironment through their activities. Thus, the periodic assessment of microbial community structure with space and time is pre-requisite so as to understand their role in ecosystem stability and development. In addition, the ability of an ecosystem to withstand extremities may contribute to variation in microbial community structure and hence microbial diversity. The commonly used form of diversity index is Shannon Weaver index (H), which is frequently used in microbial ecology studies. Diversity index is a quantitative measure that not only accounts the existence of PLFAs richness (R) but also accounts how evenly they are distributed (evenness). Total diversity depends upon (a) the number of species or number of parts (variety component), (b) the evenness component or the distribution of relative abundance. Higher overall diversity occurs when the number of species and the evenness component are larger. The bacterial and fungal PLFAs are mostly used as the measure of relative distribution of different microbial groups based on their relative abundance of certain PLFAs [20], which differ considerably among different microbial groups [58]. The study revealed significant variation in PLFA richness, Shannon diversity index and evenness in different age series iron mine overburden spoil and nearby NF soil (Table 4).

Table 4. Shannon diversity index and Pielou's evenness index based on the distribution of 75 PLFAs in the seven different age series iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) as well as NF soil across the sites.

Site	PLFA richness (*R*)	Shannon diversity index (*H*)	Pielou's evenness index (*J*)
IB_0	49	2.726231353	0.700502887
IB_2	47	2.849070813	0.739990023
IB_4	27	2.490509005	0.755652997
IB_6	43	2.717641045	0.722546252
IB_8	49	2.794246578	0.717979342
IB_{15}	49	2.783612478	0.715246919
IB_{25}	48	2.74195037	0.708294496
NF	43	3.17054272	0.842960391

Greater PLFA richness (R) was attributed by IB_0, IB_8 and IB_8 (49) compared to other mine spoil profiles. Shannon diversity index (H) varies from 2.4905 (IB_4) to 2.8490 (IB_2) across the sites. The relative abundance of evenness is the apportionment of individuals among the species is an important component of diversity index, which quantifies how equal the community is numerically. It would be useful to assess the contribution of this component to the diverse index value. The evenness of PLFA reflects the broad-scale changes in terms of the relative dominance of certain microbial groups although an evenness index should be independent of the number of species [50]. The evenness of community otherwise called Pielous evenness index (J) is constrained between 0 and 1 represents the ratio of observed heterogeneity to maximum possible heterogeneity. The Pielous evenness index (J) based on the distribution of 75 PLFAs varies from 0.7005 (IB_0) to 0.7556 (IB_4) across the sites (Table 4). The more even the distribution of PLFAs or less variation in community between microbial groups, greater is

the microbial diversity. Thus, the value of diversity index increases when both the number of types of PLFAs and evenness increases.

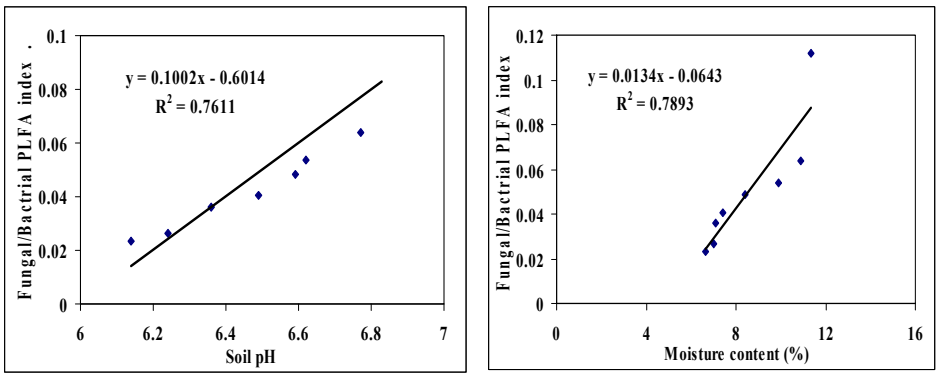
Shannon diversity index based on the distribution of different microbial groups in different iron mine overburden spoil and NF soil was calculated. Higher Shannon Weaver index in IB_2 (1.2943) revealed higher population diversity compared to IB_0 (1.2633). Comparatively higher level of microbial diversity was exhibited by IB_6 (1.1378) than IB_4 (1.1228). Higher level of microbial diversity was exhibited by IB_{15} (1.1643) compared to IB_8 (1.1341). However, IB_{25} (1.1069) exhibited relatively lower microbial diversity compared to IB_8 and IB_{15}, which indicated that the microbial communities in less disturbed ecosystem like IB_{25} may be dynamic in terms of functional responses to perturbation but more resistance to changes in microbial community composition [56]. The shift in microbial community structure and diversity among different iron mine overburden spoil 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 microbial biomass and used as functional index of the soil subsystem [80].

E. Fungal: bacterial biomass ratio

Fungal biomass was calculated based on the relative distribution of PLFA 18:2ω6c across the sites. Total bacterial biomass was obtained by summation of the distribution of PLFAs 14:0, 15:0, a15:0, i15:0, i16:0, 16:1ω7c, 16:1ω11c, 10Me 16:0, 17:0, a17:0, cy17:0, i17:0, 17:1ω8c, 10Me 17:0, 18:0 2OH, 18:1ω5c, 18:1ω7c, 10Me 18:0, 19:1ω6c and cy19:0ω8c. An index of fungal to bacterial (F/B) ratio of microbial biomass was used to study the state of microbial community in response to different environmental stresses [50]. The F:B ratio was suggested as the potential tool to discriminate the disturbed from undisturbed soil system [20, 71, 81]. The fundamental difference in bacterial/fungal physiology and ecology would suggest that the biogeography of each group would be influenced by different edaphic factors, which may vary among different soil profiles [82]. The bacteria and fungi are likely to have distinct functional roles in different mine spoil profiles and therefore more robust understanding of the site-specific effects of land use patterns and edaphic factors on these microbial population will improve the ability to predict changes in microbial community composition and function [50]. The F:B ratio exhibited an increasing trend from IB_0 (0.0233) to IB_{25} (0.0640) over time. Comparatively higher F:B ratio was estimated in IB_{25} (0.0640) compared to IB_2 (0.0263), IB_4 (0.0362), IB_6 (0.0405), IB_8 (0.0485) and IB_{15} (0.0538). However, the difference in F:B ratio in chronosequence iron mine overburden spoil was less pronounced due to heavy metal contaminated extreme environmental conditions [13, 24, 70]. Highest F:B ratio was observed in NF soil (0.1116) compared to different age series iron mine overburden spoil, which may be due to higher prevalence of fungal PLFAs exhibiting higher C:N ratio and low bulk density [83]. The bacterial PLFAs increased considerably with the increase in pH and were found to be higher in NF soil [84]. However, fungal biomass is higher in acid soil with high C:N ratio indicating that pH appears to be the most important factor for microbial abundance, diversity and activities [55, 84-85]. In fact, soil pH affects microbial processes such as organic matter mineralization, which is slowed down or even stopped at higher acidic or alkaline pH [86-87]. Besides, the shift in microbial community may also be related to the capacity of fungi for translocation N to C availability or direct influence of N supply on plant belowground C allocation is thought to be important in NF soil with higher C:N ratio [81, 84]. Several investigations have suggested that the level of activity and size of microbial communities are C limited [88]. Further, higher F:B ratio in NF soil can be explained on the basis of the existence of higher relative distribution fungal PLFAs (2.74%) compared to different iron mine overburden spoil. In addition, NF soil was supported with distinct microbial communities that can be correlated with factors that define the land-use pattern and associated soil quality influencing microbial community composition [56]. It is evident that NF soil appeared to be set apart from other mine spoil profiles with higher abundance of arbuscular mycorrhizal fungi (2.13%), which may be better able to cope with available N and organic matter. These parameters show linear increase with increased abandonment duration consistent with fungal to bacterial ratio [81, 84, 89-90]. The study indicated that the disturbed ecosystems have lower F:B ratio whereas organically managed soil have increased F:B ratio than conventional system [91].

Further, the changes in microbial community structure may be influenced by spatial variability in soil physicochemical properties (pH, moisture and nutrient availability), which influence microbial transformations altering nutrient cycling useful in providing insight how these microbes could affect the soil quality status. Differences in soil pH can arise due to the variation in vegetation pattern and management regimes. Thus, soil pH serves as one of the integrating variables and reasonably good predictor of microbial community composition [92-93]. The decline in soil pH from NF soil to IB_0 may be one of the major constraints/stresses that shift the microbial community structure [51, 67]. Comparative analysis of F:B ratio suggested that lower pH resulted decline in PLFAs (a15:0, 16:1ω5c, 18:1ω7c, 18:1ω9c, 18:2ω6c, 18:3ω6c) in IB_0 compared to undisturbed NF soil [55]. The decreased stress with gradual improvement in soil pH towards neutral in NF soil in chronosequence iron mine overburden spoil could be related to an increase in F:B ratio due to nutrient availability leading to the shift in microbial community structure [55, 67, 84, 94-95]. The study revealed positive correlation between F:B ratio and soil pH ($r = 0.966$, $p < 0.001$), which suggested that soil pH can

account 93.47% of the variability in F:B ratio (Figure 3a). Besides, the shift in microbial community as well as F:B ratio may be due to variation in moisture content from IB_0 to NF soil, which influence osmotic potential, transport of nutrients regulating microbial mediated processes and competitive interactions between microbial species [66, 96-97]. Soil moisture content exhibited positive correlation with F:B ratio ($r = 0.784$, $p < 0.001$), which can account 61.48% of the variability in F:B ratio across the sites (Figure 3b). The changes in moisture content can alter microbial community composition and function due to differences in drought tolerance among taxonomic and functional groups of soil microorganisms [98-99].



(a) (b)

Figure 3. Correlation between fungal to bacterial ratio with (a) soil pH and (b) moisture content in the different age series iron mine overburden spoil and NF soil.

F. Cluster analysis

Relative distributions of 75 PLFAs among different age series iron mine spoil and NF soil profiles were subjected to cluster analysis based on distance matrix revealed the existence of seven clusters (I-VII) (Figure 4). The dendrogram revealed highest similarity (71.8998) between IB_0 and IB_6 (cluster VII). The relatedness between IB_0 and IB_8 (cluster-VI), IB_0 and IB_{15} (cluster-V) and IB_0 and IB_2 (cluster-IV) exhibited similarity level 67.1458, 56.7162 and 52.6909 respectively. The similarity level between IB_4 and IB_{25} was estimated to be 49.0519 (cluster-III). IB_0 and IB_4 exhibited similarity level (48.3590) representing cluster-II. Minimal similarity level (34.7702) was observed between IB_0 and NF (cluster-I). The study indicated that the seven clusters based on relative distribution of 75 PLFAs exhibited the tree likeness of original (unrandomized) tree was statistically well resolved (Figure 4).

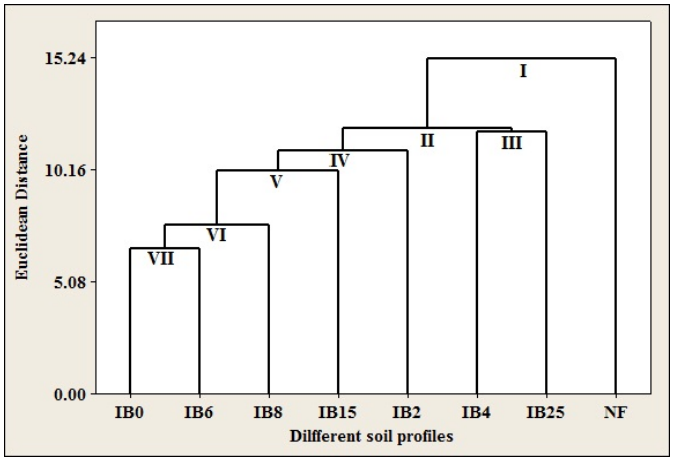


Figure 4. Cluster analyses illustrated the relatedness based on the relative distribution of 75 PLFAs among different age series iron mine overburden spoil as well as NF soil.

Further, principal component analysis was performed in order to discriminate seven different age series iron mine overburden spoil and nearby NF soil based on the relative distribution of 75 PLFAs across the sites (Figure 5) [100]. Eigen vectors determine the direction of maximum variability specifying the variances. Principal component analysis suggested that the Z1 and Z2 components explained maximum variance with their cumulative percentage of variance estimated to be 58.57%. The relative distribution of 75 PLFAs revealed differential microbial community structure among seven different age series iron mine overburden spoil in chronosequence and nearby NF soil were well segregated (Figure 5).

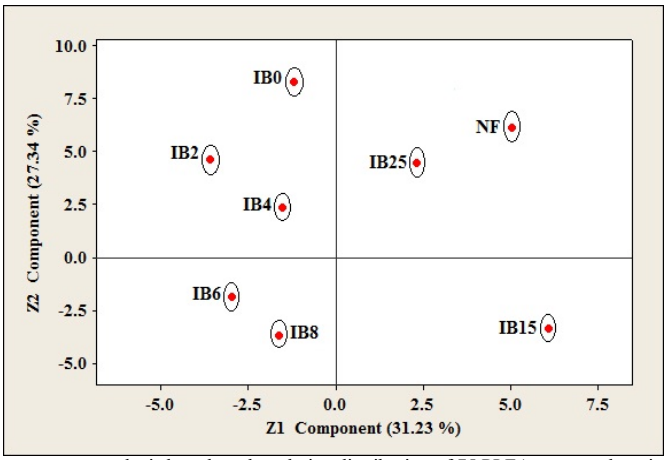


Figure 5. Principal component analysis based on the relative distribution of 75 PLFAs among the microbial communities in different mine overburden spoil as well as NF soil.

G. Multivariate analyses

Redundancy analysis was used to examine the patterns in PLFA data collected from different age series iron mine overburden spoil reflecting relationship between different mine spoil profiles, species and environmental gradients altogether. The changes in microbial community structure may occur in response to altered physico-chemical properties that affect the soil microenvironment with possible effects on the efficiency of readily mineralizable resource conservation by soil microbes. RDA analysis allowed examining the variation in PLFA patterns in terms of both iron mine overburden sites and the measured environmental gradients including enzyme activities, which was found to be significant ($p < 0.005$). A total of 61.62% of the variability could be explained based on the fitted PLFA data by the model from the canonical sum of eigen values. Seven different age series iron mine overburden sites and environmental gradient arrows for the RDA ordination of PLFA data were shown (Figure 6a). The slit and clay %, moisture content (MC), water holding capacity (WHC), pH, organic C (OC), total N (TN), extractable P (EP) and enzyme activity (amylase, invertase, protease, urease and dehydrogenase) increased in the general direction of IB_{25}, while sand % and bulk density (BD) increased towards IB_0. The increasing trend of these parameters correspond to enhance accumulation of organic C, available N and microbial community composition as vegetation succession proceeded over time reflecting the sign of mine spoil restoration [101-103].

The data related to physico-chemical properties and enzyme activities in chronosequence iron mine overburden spoil were taken for RDA analysis [67, 83]. The proportions of certain PLFAs were highly correlated with physico-chemical properties (Jackson *et al.*, 2003) in different iron mine overburden spoil over time (Figure 6b). The clay, pH, MC, WHC, OC, TN, EP and enzyme activities were highly correlated with PLFAs (a14:0, 14:1ω7c DMA, 14:1ω8c, 16:1ω5c, 16:1ω7c, 18:1ω7c, 18:1ω9c, 18:2ω6c, 18:3ω6c, 21:1ω8c and 24:0), while sand and BD with PLFAs (12:1ω8c, 14:1ω5c, a15:0, 16:0 aldehyde, 16:0 2OH, 16:2 DMA, 10Me 18:0, 18:1ω7c DMA, 19:0cy ω7c and 10Me 19:1ω7c) across the sites. Further, the negative correlation coefficients indicated that the changes in microbial community structure in response to disturbances were associated with the decrease in respective soil properties in different mine spoil profiles. Although 75 PLFAs were included in RDA ordination, but the PLFAs with highest species scores in the first two ordination axes, which correlated well with environmental variables and important biological markers are displayed for clarity (Figure 6b). Some general patterns emerge from this analysis. The existence of higher level of methyl-branched PLFAs (10Me16:0; 10Me22:0) and saturated branched fatty acids (C_{16} to C_{19}) in IB_0 suggested higher relative abundance of actinomycetes and anaerobic bacteria respectively. In addition, higher pyrite (FeS_2) contamination provides suitable condition for the existence of PLFA a17:0 reflecting higher distribution of sulfate reducing bacteria in IB_0. Further, the level of saturated branched fatty acids (C_{14} to C_{16}) was found to be comparatively higher in IB_{10} than different age series iron mine overburden spoil suggesting higher relative abundance of gram-positive bacteria in IB_{10}. Minimal longer chain PLFAs in IB_0 indicated comparatively lower input from microeukaryotes, which may be influenced by acidic pH and induced toxic metal contamination [57, 94, 105]. Higher relative abundance of arbuscular mycorrhizal fungi (16:1ω5c) and heterotrophic microeukaryotes were observed in IB_{25}. Higher level of fungal PLFAs 18:3ω6c and PLFAs 18:1ω9c, 18:1ω5c, 18:1ω7c suggested higher relative distribution of fungal population in IB_{25} and IB_{15} respectively. Besides, higher level of PLFAs 16:1ω5c and 16:1ω7c suggested higher relative distribution of aerobic bacteria in IB_{25} and IB_{15} respectively. The study suggested that the shift in microbial community structure from IB_0 to IB_{25} may be attributed to the change in soil quality in the direction of IB_{25} supplementing the mine spoil restoration over time.

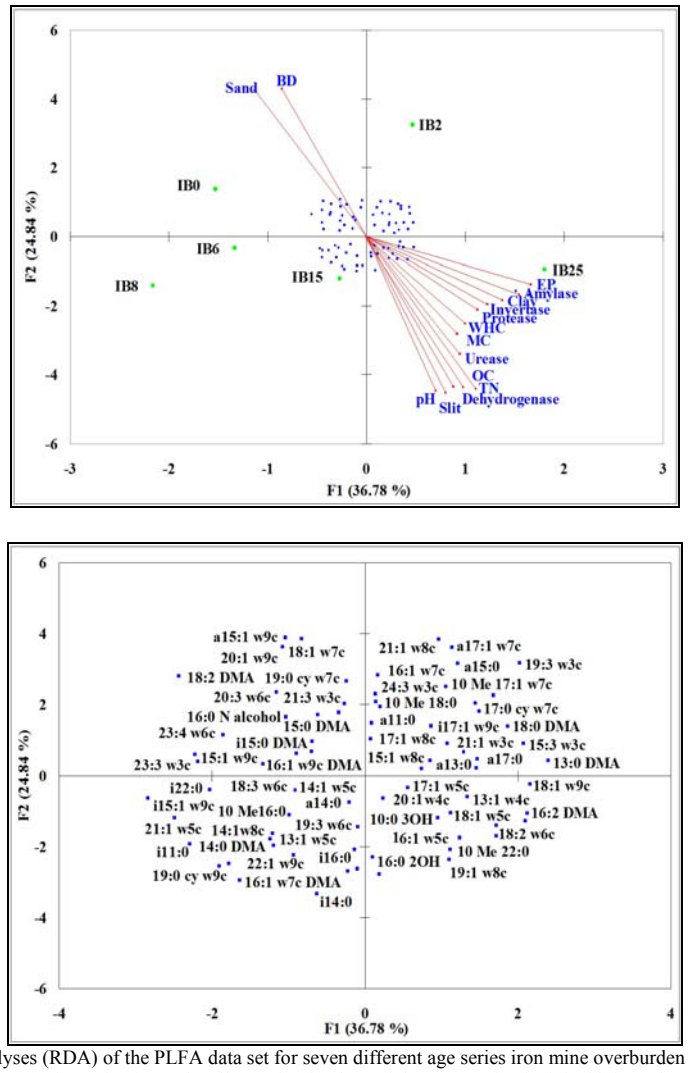


Figure 6. Redundancy analyses (RDA) of the PLFA data set for seven different age series iron mine overburden spoil, using 75 PLFAs and 11 environmental variables. (a) Site codes for each soil sample; (b) showed the PLFAs that had the highest absolute species scores on each of the two axes, along with additional PLFAs of biological interest.

IV. CONCLUSION

The changes in microbial community structure not only ascertain the microbial diversity, but also the function and nature of interactions among existing microbial species as well as the physiological state of ecosystem. A realistic ecological assessment of iron mine spoil restoration implies periodic monitoring. The presence and abundance of these signature fatty acids in mine spoil revealed the presence and abundance of a particular microbe or groups of microbes. PLFA analysis can be used for comparative assessment of physiological status of microbial communities in different mine spoil profiles. The multivariate analysis revealed that seven different age series iron mine overburden spoil had distinctly different PLFAs and microbial community composition. The spatial and statistical results of PLFA analysis revealed the pace or rapidity of alteration in microbial community structure. Further, the changes in microbial community composition may occur in response to altered physico-chemical properties with possible effects on the efficiency of C conservation mediated by soil microorganisms. Nevertheless, the readily mineralizable source of organic matter would enhance microbial processes including enzyme activities to change microenvironment. PCA revealed that the microbial communities were compositionally distinct. Thus, PLFA profiling provides a sensitive and meaningful measure of microbial community composition to monitor mine spoil restoration based on soil quality assessment in chronosequence iron mine overburden spoil over time compared to undisturbed NF soil.

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