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Contribution of Soil Physico-Chemical Properties Influencing Microbial Biomass Used as Biomarkers for Mine Spoil Genesis.

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ABSTRACT

Iron mining activities lead to upsetting the soil biological equilibrium through the generation of mine spoil dumped in the form of overburden has become a major environmental concern. The burgeoning concern about the slow recovery of mine spoil have emphasized inculcation about the periodic monitoring of mine spoil genesis through different soil quality indicators, which evaluates the degree of functional microbial processes for ecosystem restoration. Being the reliable and sensitive indicators to assess the degree of restoration, the level of microbial biomass pool in different mine spoil samples ranges from MB-C (51.324 - 593.789 µgC/g spoil), MB-N (4.428-61.149 µgN/g spoil), and MB-P (2.216-27.392 µgP/g spoil). Greater proliferation of microbial biomass pool over a period of 25 years revealed mine spoil genesis supporting the sign of restoration. Stepwise multiple regression analysis was performed to determine the contribution of physico-chemical properties influencing the variability in microbial biomass pool across the sites. Principal component analysis can able to discriminate seven iron mine overburden spoil and nearby forest soil into independent clusters. Further, the redundancy analysis suggested that different age series iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) with distinct microbial communities are influenced by the physico-chemical properties.

Keywords: Iron mine spoil, microbial biomass, organic C, physico-chemical properties.

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INTRODUCTION

Iron mining activities scars the landscape and disrupts ecosystem with generation of large quantities of mine spoil with metal contaminations heaped in the form of overburden leading to nutritionally deprived habitats. Being deficient in plant nutrient due to the lack of biologically rich surface soil, mine overburden spoil represents a disequilibrated geomorphic system [1,2], poses problem for pedogenesis and restoration [2,3]. Slow recovery process of mine spoil was reported due to poor physico-chemical properties [2] as well as different biological constraints associated with microbial growth [4,5], and vegetational succession [1,6,7]. Further, long term metal contamination in mine spoil disrupts ecosystem structure and functions through alternation in microbial activity [2,8].

Soil microbial community structure is an inherent component of soil quality assessment for ecosystem functioning as the microbes play a vital role in carbon flow, organic matter decomposition, and nutrient cycling [9,10]. However, the iron mine spoil created in the aftermath of mining activities represents rigorous conditions and supplements profound effects in shifting of microbial community structure and function that mediates changes in biogeochemical processes in terrestrial ecosystem [2]. The importance of understanding ecosystem functioning has led to an increased interest for assessment of mine spoil genesis and soil quality, which does not adequately reflected by physico-chemical properties alone [11]. Besides, microbial biomass is considered as more dynamic soil quality parameter than those based on physico-chemical properties, because of its rapid turnover as compared to other components of organic matter in soil [8]. Nevertheless, microbial biomass indices were frequently used as early indicators of changes in physico-chemical properties resulting from soil management, anthropogenic activities and environmental stresses [12]. Therefore, the periodic estimation of biologically active fractions of organic matter such as microbial biomass is pre-requisite to reflect changes in mine spoil genesis that alter nutrient dynamics in chronosequence iron mine overburden spoil over time.

Soil microbial biomass is the active component of soil organic pool that constitutes a transformation matrix for organic matter. Assimilation and liberation of nutrients by and from the biomass is supposed to make a considerable contribution to plant nutrition, and hence the microbial biomass is considered both as source and sink of nutrients [13]. Size of microbial biomass pool is considered to be the functional index of soil quality [3,10], ecological marker of soil health [8], a labile pool of plant nutrients [7,9] that determine microbial community to support the nutrient conserving mechanism through immobilization and mineralization [14]. Thus, the assessment of microbial biomass, activity, and nutrient levels has attracted considerable attention [6,9,15].

Soil microbial biomass-carbon comprises 1-5% of total organic carbon [14,16]. Because of its high turnover rate, microbial biomass-carbon could respond more rapidly to changes of soil environment than soil organic matter [13]. Microbial biomass-carbon acts as 'living engine' maintaining soil fertility that protects soil from contamination and degradation [4,5]. Besides, it is also used to determine the effect of toxic substance on soil microbial community [17]. Microbial biomass constitutes a significant part of the potentially mineralizable nitrogen, and serves both as the transformation agent and source-sink of nitrogen [9,11,18]. Consequently, the assessment of microbial biomass-nitrogen has importance in quantifying the nitrogen dynamics that reflects nitrogen availability and overall nitrogen cycling in terrestrial ecosystem [11,18]. Microbial biomass-phosphrous accounted 2–10% of total phosphrous [19]. The rapid turnover of phosphorous in microbial pool may contribute a major source to available phosphorous pool, as phosphorous is released from microbial biomass is highly available to plant uptake, and also the microbial immobilization of inorganic phosphrous protects the phosphorous from physico-chemical fixation [2,11].

Assessment of microbial biomass provides a sensitive indicator of the changes brought about by soil management; long before such changes can be detected in soil organic carbon [11,13,14]. The percentage of organic carbon reflected in microbial biomass (MB-C:OC) provides an insight into the soil organic carbon status and functional index of soil subsystem [5,13]. Assessment of microbial biomass can be considered as the quantitative measure of soil degradation and mine spoil genesis supporting reclamation progress [4,6,16] due to its contribution towards nutrient flow, organic matter turnover and soil structural stability in soil management and perturbation studies [5,20]. In addition, the microbiological parameters have been proposed as sensitive and reliable indicators of stresses or perturbations in soil affected by mining wastes [2,16]. However, the microbial biomass is being directly and indirectly influenced by clay content [1,20], hydrological regimes and vegetation [2,3,7]. Therefore, the contribution of physico-chemical properties affecting microbial biomass in chronosequence was determined reflecting mine spoil genesis over time.

In view of increased mining activities and adverse effects on microbial community, it is the utmost concern to characterize the drastically altered soil properties of different age series iron mine spoil, which pave the way of greater understanding in the direction of improving soil quality. The successful rehabilitation of mine spoil requires understanding of the progress of mine spoil genesis influenced by different soil physico-chemical properties and microbial biomass indices. However, the data related to physico-chemical and microbial biomass dynamics in iron mine spoil were scanty. Based on the facts, the present study was designed to assess the impact of different soil attributes on nutrient cycling in order to determine the progress of mine spoil genesis with respect to seven different age series iron mine overburden spoil (fresh to 25 yr old) in chronosequence by determining the degree of variability in microbial biomass (MB-C, MB-N, and MB-P), as well as through certain integrating quotients (MB-C:OC, MB-C:MB-N, and MB-C:MB-P) over time.

MATERIALS AND METHODS

Study Site

The study was carried out in iron mining area located at Noamundi in the revenue district of West Singhbhum, Jharkhand, India (Geographical location: 85° 28' 02.61" east longitude and 22° 8' 33.93" north latitude). The study site is situated away from the mean sea level *i.e.* about 581 m altitude. The area experiences a semi-arid climate with annual rainfall of 1340 mm yr^{-1}, mean annual average temperature 19.67°C, and humidity 20%. The study site experienced three distinct seasons *i.e.* summer (April), rainy (July) and winter (January). Tropical dry deciduous forest is considered to be the natural vegetation of the area. However, extensive iron mining activities led to decline of forest cover, and generated a number of iron mine overburden. Seven iron mine overburdens in chronosequence have been selected based on the time elapsed since inception including 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}) respectively.

Mine Spoil Sampling

Mine spoil samples were collected from seven age series iron mine overburdens (IB_0, IB_2, IB_4, IB_6, IB_8, IB_{15} and IB_{25}) within a peripheral distance of 10 km from the core iron mining area. Besides, the nearby native forest soil (NF) was selected adjacent to core mining area for comparison. 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 individually from different age series iron mine overburden. The samples were subjected to sieving and stored at 4°C.

Physico-Chemical Characterization

Soil textural analysis includes the estimation of clay (0.002 mm), slit (0.06-0.002 mm) and sand (2.0-0.06 mm) percentage. Bulk density was determined as per the procedure described in TSBF Handbook [21]. The moisture content and water holding capacity was estimated [22]. Soil pH (1:2.5 ratio of soil: water) was measured with digital pH meter. Soil organic C was estimated through titration method suggested by Walkley and Black [22]. Total N was determined following Kjeldahl method [23]. The extractable P was estimated using chlorostannous reduced molybdophosphoric blue colour method [24].

Microbial Biomass-C, N And P

Microbial biomass C (MB-C) was determine by fumigation extraction method [25] by back titration with 0.04 $(NH4)_2Fe\ (SO_4)_2.6H_2O$ using ferroin indicator taking $E_C/K_{EC} = E_C/0.38$; where EC = difference in organic C extracted from fumigated and unfumigated sample; K_{EC} = calibration factor (0.38). Microbial biomass N (MB-N) was estimated using $CHCl_3$ fumigation method [26] taking the value of K_N (fraction of biomass N extracted after fumigation) factor 0.54. Microbial biomass P (MB-P) was estimated using the procedure where inorganic P was extracted by 0.5M $NaHCO_3$ solution adjusted to pH 8.5 with NaOH [27]. Extracted P was measured by $CHCl_3$ fumigation method [19]. MB-P was calculated using $NaHCO_3$ extractable P from fumigated sample minus that extracted from unfumigated sample, then divided by K_P (Fraction of biomass P extracted after fumigation) value of 0.40.

Statistical Analysis

Simple correlation analysis was conducted to test the level of significance between different soil properties using SPSS (version 17.0). Stepwise multiple regression analysis was employed to quantify the contribution of different physico-chemical properties explaining the variability in microbial biomass C, N, and P using Minitab 16 software. Principal component analysis was performed using Statistrix PC DOS Version-2.0 (NH Analytical software). Redundancy analysis was performed using Microsoft Excel XLSTAT-2014 (Version 2.03).

RESULTS AND DISCUSSION

PHYSICO-CHEMICAL CHARACTERIZATION

Table 1. Physico-chemical characterization of seven different age series iron mine overburden spoil (IB$_0$ → IB$_{25}$) and nearby NF soil across the sites.

Parameters	Different age series iron mine overburden spoil							Forest soil (NF)
	IB$_0$	IB$_2$	IB$_4$	IB$_6$	IB$_8$	IB$_{15}$	IB$_{25}$	
Sand (%)	87.8 ± 2.15	85.9 ± 1.84	84.8 ± 1.75	83.4 ± 1.89	81.5 ± 1.64	79.7 ± 1.55	75.3 ± 1.36	72.5 ± 2.05
Slit (%)	7.8 ± 0.45	8.4 ± 0.33	9.1 ± 0.41	9.9 ± 0.29	10.9 ± 0.37	11.8 ± 0.41	13.5 ± 0.52	14.2 ± 0.66
Clay (%)	4.4 ± 0.33	5.7 ± 0.21	6.1 ± 0.28	6.7 ± 0.34	7.6 ± 0.38	8.5 ± 0.29	11.2 ± 0.24	13.3 ± 0.31
Bulk density (g/cm^3)	1.852 ± 0.036	1.794 ± 0.029	1.715 ± 0.035	1.664 ± 0.028	1.593 ± 0.034	1.405 ± 0.033	1.332 ± 0.029	1.259 ± 0.021
WHC (%)	24.501 ± 1.235	26.422 ± 1.558	28.067 ± 2.013	32.311 ± 2.152	37.457 ± 1.942	40.338 ± 1.675	44.509 ± 2.045	46.648 ± 2.164
Moisture (%)	6.643 ± 0.206	6.985 ± 0.211	7.106 ± 0.198	7.422 ± 0.201	8.391 ± 0.168	9.915 ± 0.176	10.886 ± 0.155	11.329 ± 0.198
Soil pH	6.14 ± 0.08	6.24 ± 0.06	6.36 ± 0.05	6.49 ± 0.06	6.59 ± 0.05	6.62 ± 0.06	6.77 ± 0.08	6.83 ± 0.08
Organic C (%)	0.142 ± 0.029	0.218 ± 0.024	0.284 ± 0.028	0.355 ± 0.034	0.815 ± 0.039	1.648 ± 0.041	2.228 ± 0.045	2.469 ± 0.052
Total N (%)	0.004 ± 0.001	0.007 ± 0.002	0.011 ± 0.004	0.015 ± 0.003	0.053 ± 0.002	0.125 ± 0.005	0.187 ± 0.007	0.245 ± 0.008
Extractable P (µg P/g spoil)	70.445 ± 2.304	76.836 ± 3.442	84.552 ± 2.987	91.707 ± 3.416	112.542 ± 8.588	645.817 ±11.508	945.678 ± 15.647	1091.509 ± 25.551

Values expressed in mean ± SD; n = 3.

Table 2. Microbial biomass- C, N and P in seven different age series iron mine overburden spoil (IB$_0$ → IB$_{25}$) and nearby NF soil across the sites.

Parameters	Different age series iron mine overburden spoil							Forest soil (NF)
	IB$_0$	IB$_2$	IB$_4$	IB$_6$	IB$_8$	IB$_{15}$	IB$_{25}$	
MB-C (µg/g spoil)	51.324 ± 3.641	72.943 ± 6.084	91.657 ± 6.358	111.758 ± 8.647	248.977 ± 12.109	472.489 ± 11.588	593.789 ± 13.427	648.719 ± 16.522
MB-N (µg/g spoil)	4.428 ± 0.312	6.452 ± 0.411	8.312 ± 0.451	10.787 ± 0.512	24.524 ± 2.116	46.992 ± 3.148	61.149 ± 4.416	68.211 ± 3.541
MB-P (µg/g spoil)	2.216 ± 0.249	3.187 ± 0.257	4.011 ± 0.261	5.075 ± 0.311	11.347 ± 0.528	21.689 ± 0.519	27.392 ± 1.108	30.728 ± 1.227

Values expressed in mean ± SD; n = 3.

Physico-chemical characterization of seven different age series iron mine overburden spoil (IB$_0$→IB$_{25}$) indicated an increasing tend in clay% that varies from 4.4% (IB$_0$) to 11.2% (IB$_{25}$), where as the sand% exhibited a reverse trend (Table 1). The nearby NF soil accounted 72.5% sand, 14.2% slit, and 13.3% clay respectively. Gradual establishment of vegetation cover may be the reason for such increase in clay% across the sites [1,20]. Textural composition of mine spoil influences different hydrological regimes. Bulk density exhibited a decline trend *i.e.* from IB_0 (1.852 g/cm^3) to IB_{25} (1.332 g/cm^3) due to the gradual increase in clay percentage (r = -0.962; $p < 0.01$) (Table 2), and devoid of vegetation in IB_0 [2]. Bulk density exhibited by IB_{25} showed closer resemblance with nearby NF soil (1.259 g/cm^3) due to the accumulation of clay fraction and organic matter supported by vegetation promoting macro-aggregation [20]. However, water holding capacity showed reverse trend, which ranges from 24.501% (IB_0) to 44.509% (IB_{25}).

Similar trend was exhibited with respect to moisture content *i.e.* minimum in IB_0 (6.643%) and maximum in IB_{25} (10.886%). Gradual increase in clay% was found to be positively correlated with WHC (r = 0.953; $p < 0.01$), and MC (r = 0.964; $p < 0.01$) (Table 3). Relatively higher water holding capacity (46.648%) and moisture content (11.329%) in nearby NF soil was observed as compared to different iron mine spoil, which may be due to dense vegetation cover. Besides, the progressive improvement in pH that varies from 6.14 (IB_0) to 6.77 (IB_{25}) was observed across the sites. Acidification of mine spoil was reported to be contributed by minerals deposition [1], oxidation of iron and sulphur compounds and accumulation of organic C leading to the formation of organic acids over time [6,13].

Table 3. Simple correlation coefficients among soil physico-chemical variables and microbial biomass pool in seven iron mine spoil ($IB_0 \rightarrow IB_{25}$) and nearby NF soil.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13
X1	1												
X2	-0.994**	1											
X3	-0.996**	0.979**	1										
X4	0.979**	-0.990**	-0.962**	1									
X5	-0.975**	0.992**	0.953**	-0.986**	1								
X6	-0.977**	0.983**	0.964**	-0.989**	0.976**	1							
X7	-0.963**	0.981**	0.940**	-0.967**	0.984**	0.935**	1						
X8	-0.972**	0.972**	0.963**	-0.978**	0.959**	0.997**	0.912**	1					
X9	-0.973**	0.960**	0.975**	-0.959**	0.936**	0.982**	0.888**	0.991**	1				
X10	-0.942**	0.929**	0.944**	-0.940**	0.897**	0.968**	0.843**	0.982**	0.987**	1			
X11	-0.968**	0.973**	0.955**	-0.982**	0.965**	0.999**	0.917**	0.999**	0.984**	0.974**	1		
X12	-0.972**	0.975**	0.961**	-0.981**	0.964**	0.998**	0.917**	0.999**	0.989**	0.978**	0.999**	1	
X13	-0.970**	0.974**	0.959**	-0.982**	0.965**	0.999**	0.917**	0.999**	0.987**	0.976**	0.999**	0.999**	1

** Correlation is significant $p < 0.01$, and * correlation is significant $p < 0.05$.
Xi (i = 1-13) stands for sand, slit, clay, bulk density, water holding capacity, moisture content, pH, organic C, total N, extractable P, microbial biomass C, N, and P.

The organic C is directly linked with soil organic matter, and hence considered as an index relating to productivity of mine overburden spoil over time [2,7]. Progressive improvement in organic C (0.142 - 2.228)%, total N (0.004 - 0.187)%, and extractable P (70.445 - 945.678) μg P/g spoil with minimum in IB_0 and maximum in IB_{25} were observed over time (Table 1). However, the organic C, total N and extractable P in nearby NF soil were found to be 2.469%, 0.245% and 1091.509 μg P/g spoil respectively. Gradual improvement in organic C from a nutrient deficient iron mine spoil to an enriched NF soil over time, which may be due to the establishment of vegetation [3,28,29], input of litter from vegetation compartment and its decomposition during the course of passive or active restoration [7]. Being a source and sink of nutrients and organic content [20], the variation in clay percentage exhibited positive correlation with organic C (r = 0.963; $p < 0.01$) in ecologically derelict mining areas was substantiated by several workers [2,3]. Nitrogen is one of the major soil limiting nutrients influencing plant productivity, which is derived from organic matter, fertilizers and leguminous plants. Higher values of total N in nearby NF soil was due to the application of fertilizers, where as lower value in IB_0 was attributed to lower rates of mineralization process in fresh iron mine spoil [3]. Phosphorous is an essential element classified as macronutrient because of relatively large amounts of phosphorous is required by plants. Lower extractable P in IB_0 may be due to its presence in insoluble state, lack of organic matter, and poor mineralization process in fresh iron mine overburden spoil. The gradual accumulation of soil nutrients from IB_0 to IB_{25} may be attributed to the successional establishment of vegetation, plant species capable o nitrogen fixing potential, as well as development of mycorrhiza and other nutrient immobilizing microbial colonization [7,9,19].

MICROBIAL BIOMASS C, N AND P

The microbial biomass C, N and P exhibited a wide variation with respect to chronosequence of iron mine overburden spoil (Table 2). The variation in microbial biomass C, N, and P showed a rage of (51.324 – 593.789) μg/ g spoil, (4.428 – 61.149) μg/ g spoil, and (2.216 – 27.392) μg/ g spoil respectively. However, the MB-C (648.719 μg/ g spoil), MB-N (68.211 μg/ g spoil), and MB-P (30.728 μg/ g spoil) in nearby NF soil was found to be relatively higher as compared to different age series iron mine overburden spoil.

Minimal level of microbial biomass C, N, and P in IB_0 was observed as compared to different age series mine overburden spoil, which may be due to the hostile environment with nutrient deficient condition, heavy metal contamination and lack of vegetation cover [2,3]. Besides, the progressive increase in microbial biomass pool size with the increase in age of the iron mine overburden spoil was substantiated by several workers [2,3,7,9]. The relationship between age of mine overburden spoil in chronosequence and MB-C ($r = 0.974$; $p < 0.001$), MB-N ($r = 0.977$; $p < 0.001$), and MB-P ($r = 0.974$; $p < 0.001$) were observed to be positive and statistically significant. The study indicated the level of MB-C, MB-N and MB-P proliferated approximately 11.5, 13.8, and 12.3 times within a span of 25 years respectively. Microbial biomass proliferation is considered to be the net resultant of microbial immobilization. Greater proliferation of MB-N over MB-C and MB-P with the increase in age of iron mine spoil indicated relatively faster nitrogen immobilization during mine spoil genesis supporting the sign of reclamation [10].

The magnitude of MB-N increase appeared to be dependent on MB-C and MB-P ($r = 0.999$; $p < 0.01$) (Table 3). Greater proliferations of soil microbes over time play an important role in pedogenesis, improving root growth and amelioration of microclimatic conditions [4]. Therefore, the contribution of soil microbial biomass towards nutrient flow, organic matter turnover and soil structural stability have led the microbial ecologist to use it as biomarker for soil management practices and perturbation studies [2,3,6].

Integrating Quotients

The progress of mine spoil genesis supporting reclamation was estimated using different integrating quotients (MB-C:OC; MB-C:MB-N, and MB-C:MB-P). The ratio of microbial biomass nutrients to soil nutrients (MB-C:OC) represents the quantum of soil nutrients reflected in microbial biomass [13]. Besides, MB-C:OC provides an insight into the soil organic carbon status, proposed as useful index to monitor soil pollution by metal contamination [4], and functional index of soil subsystem [8]. In the present study, the MB-C:OC ratio ranged from 3.61 (IB_0) to 2.66 (IB_{25}) with respect to chronosequence of iron mine overburden spoil (Table 4). However, MB-C: OC ratio in NF soil was found to be 2.62. Higher MB-C:OC ratio in IB_0 suggested that the microorganisms are under stress in metal contaminated mine spoil and are less efficient for organic carbon utilization. Besides, the data indicated time-dependent decrease in MB-C:OC ratio in chronosequence iron mine overburden spoil reflecting changes in carbon mineralization rate as the level of metal contamination in mine spoil decreased with the gradual improvement in organic carbon supplemented by vegetation [7]. Progressive decrease in MB-C:OC ratio in different age series iron mine overburden spoil in chronosequence indicated the success of reclamation efforts, but steady state is yet to be achieved [8]. The average value of MB-C:OC (2% to 4%) reported by several workers [15] substantiated the concept. The variation in organic carbon was positively correlated with MB-C ($r = 0.999$; $p < 0.001$) across the sites (Table 3), which corroborates with several studies [2].

Table 4. Integrating quotients including MB-C:OC, MB-C:MB-N, and MB-C:MB-P ratio in different age series iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) and nearby NF soil.

Soil profiles	MB-C : OC	MB-C : MB-N	MB-C: MB-P
IB_0	3.61	11.59	23.16
IB_2	3.34	11.30	22.88
IB_4	3.22	11.02	22.85
IB_6	3.14	10.36	22.02
IB_8	3.05	10.15	21.94
IB_{15}	2.86	10.05	21.78
IB_{25}	2.66	9.71	21.67
NF	2.62	9.51	21.11

Microbial C:N ratio reflects microbial community structure that varied with the concentration gradients *i.e.* fungi dominated in polluted soil and bacteria in uncontaminated soil [29]. Relatively higher microbial C:N ratio is caused by increased fungal to microbial biomass pool [12]. The variation in microbial C:N and C:P ratio ranged from 9.71 to 11.59 (K_N = 0.54) and 21.67 to 23.16 (K_P = 0.40) respectively (Table 4). Similar range of microbial C:N ratio was reported in Trukish forest soils [11], (10.5-13.8) in certain Indian tropical forest soil [10]. However, the variation in microbial C:N ratio under different soil conditions may not always be compared, because the proportion of MB-C and MB-N mineralized in terms of K_N (fraction of biomass N released after $CHCl_3$ fumigation) and K_P (fraction of biomass P extracted after $CHCl_3$ fumigation) values may vary.

Stepwise Multiple Regression Analysis

Table 5. Stepwise multiple regression analysis of MB-C, MB-N, and MB-P on different physico-chemical variables in chronosequence iron mine overburden spoil.

Microbial Biomass Nutrients	Equation(s)	R^2
MB-C	= 21.891 + 259.3 OC = 7.965 + 333.9 OC – 768 TN = 3.79646 - 37.7 OC + 24.3 MB-P	0.997 0.999 0.999
	= 75.707 + 2606 TN = - 1.20698 – 305 TN + 23.65 MB-P	0.969 0.999
	= 69.86 + 0.556 EP = 14.52 – 0.112 EP + 310 OC = 54.56 – 0.116 EP + 336 OC – 8.3 Clay	0.949 0.998 0.999
	= - 344.151 + 79.45 Clay = 25.334 – 4.1 Clay + 22.24 MB-P	0.911 0.999
	= - 5749.708 + 927.9 pH = - 1.351 + 1.2 pH + 21.21 MB-P	0.841 0.999
	= 2029.3 – 1105 BD = - 1505.5 + 254 BD + 162 MC = 146.6 – 64 BD + 144 MC – 153 pH	0.964 0.998 0.999
MB-N	= 0.96192 + 27.35 OC	0.999
	= 6.5505 + 275.8 TN = - 0.2191 + 42.8 TN + 0.0894 MB-C	0.977 0.999
	= 5.95364 + 0.05874 EP = - 0.51327 + 0.00203 EP + 2.164 MB-P	0.955 0.999
	= - 38.04 + 8.428 Clay = - 1.8946 + 0.255 Clay + 2.176 MB-P	0.923 0.999
	= - 607.252 + 97.79 pH = - 1.288 + 0.09 pH + 2.236 MB-P	0.841 0.999
	= - 90.9472 + 13.96 MC = - 0.7665 + 0.01 MC + 2.236 MB-P	0.997 0.999
MB-P	= 0.74791 + 12.21 OC = 0.14004 – 5.07 OC + 0.632 MB-N	0.998 0.999
	= 3.252115 + 123.1 TN = 0.060251 + 13.2 TN + 0.0422 MB-C	0.975 0.999
	= 2.98809 + 0.02621 EP = 0.2483 – 0.00083 EP + 0.46 MB-N	0.952 0.999
	= - 16.6142 + 3.757 Clay = 0.833 – 0.109 Clay + 0.4587 MB-N	0.919 0.999
	= - 270.9917 + 43.69 pH = 0.1788 + 0.02 pH + 0.4466 MB-N	0.841 0.999
	= 33.527141 + 1.334 WHC = 0.00554 + 0.012 WHC + 0.443 MB-N	0.930 0.999
	= - 40.328 + 6.24 MC = - 2.773 + 0.47 MC + 0.413 MB-N	0.997 0.999

*All R^2 values are significant at $p < 0.001$.

Stepwise multiple regression analysis was performed in order to explain the contribution of different physico-chemical properties of mine spoil on microbial biomass C, N and P as dependent variables in different age series iron mine overburden spoil (Table 5). The analysis suggested that 99.7% of the variability in MB-C was explained by OC, and a marginal effect (0.2%) by TN or MB-P as 2nd variable ($p < 0.001$). Besides, MB-C is positively correlated with TN (96.9%; $p < 0.001$), and an additional 3% was contributed by MB-P as 2nd variable. Similarly, EP contributed 94.9% of the variability in MB-C. The 2nd and 3rd variables of importance were OC

(4.4%) and clay (0.1%). Similarly 91.1% variability in MB-C was explained by clay, and an additional 8.8% by MB-P as 2nd variable. With pH as 1st variable explained 84.1% of the variability in MB-C, and an additional 15.8% by MB-P as 2nd variable. Besides, BD contributed 96.4% variability in MB-C and an additional 3.4% by MC, marginal effect by pH as 3rd variable.

About 99.9% of the variability in MB-N was explained by OC ($p < 0.001$). The TN explained 97.7% variability in MB- N and a marginal effect by MB-C (2.2%) as 2nd variable. With EP as 1st variable explained 95.5% of the variability in MB-N and an additional 4.4% by MB-P as 2nd variable. Similarly clay contributed 92.3% of the variability in MB-N and an additional 7.6% by MB-P. About 84.1% of the variability in MB-N was explained by pH and an additional 5.4% by MB-P as 2nd variable. With MC as 1st variable explained 99.7% variability in MB-N and a marginal effect was contributed by MB-P as 2nd variable (Table 5). About 99.8% of the variability in MB-P was explained by OC ($p < 0.001$) and an additional 0.1% by MB-N as 2nd variable. Besides, 97.5% of the variability in MB-P was explained by TN, and an additional 2.4% by MB-C as 2nd variable. Similarly, EP contributed about 95.2% of the variability in MB-P and an additional 4.7% by MB-N as 2nd variable. With clay as 1st variable explained 91.9% of the variability in MB-P and an additional 8% by MB-N as 2nd variable. About 84.1% of the variability in MB-P was explained by pH and an additional 6.9% by MB-N as 2nd variable. About 99.7% of variability in MB-P was explained by MC, and a marginal effect by MB-N as 2nd variable (Table 5).

Principal Component Analysis

Principal component analysis was performed in order to discriminate seven different age series iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) and nearby NF soil based on their physico-chemical properties and microbial biomass C, N and P level [30]. From the values of such variables, the vectors and components ($Z_1 \rightarrow Z_{13}$) were calculated for different sites, and were ordered in terms of their eigen values and percentage of variance. Based on the eigen values, the Z_1 and Z_2 components explained maximum variance with 99% cumulative percentage of variance, which can able to segregate seven different mine overburden spoil and nearby NF soil profiles into independent clusters (Figure 1).

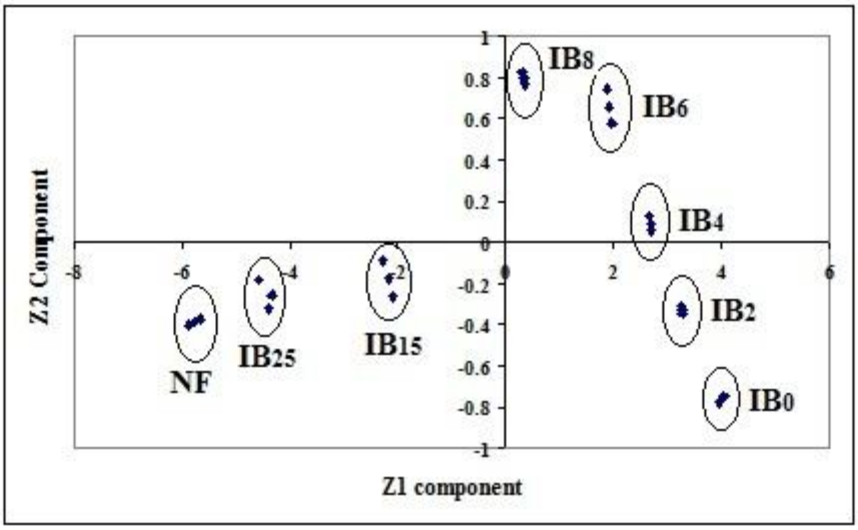

Figure 1. Principal component analysis based on physico-chemical properties and microbial biomass indices among seven iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) and nearby NF soil.

Redundancy Analysis

Redundancy analysis can able to explain the relationship between different ages series iron mine overburden in chronosequence ($IB_0 \rightarrow IB_{25}$) and the environmental gradients altogether not only in concert in same model, but also unlike discriminate analysis there is no limit on the number of variables that can be used relative to the number of samples. Changes in microbial biomass C, N, and P may occur in response to the variation in different physico-chemical attributes affecting microenvironment with possible impacts on the efficiency of readily mineralizable resource conservation by the soil microorganisms. The redundancy analysis revealed that seven different iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) had distinctly different microbial community structure (Figure 2). The changes in microbial biomass pool in different age series iron mine overburden spoil may occur in response to the altered physico-chemical variables with possible effects on the efficiency of carbon conservation by the soil microorganisms was evident form the study.

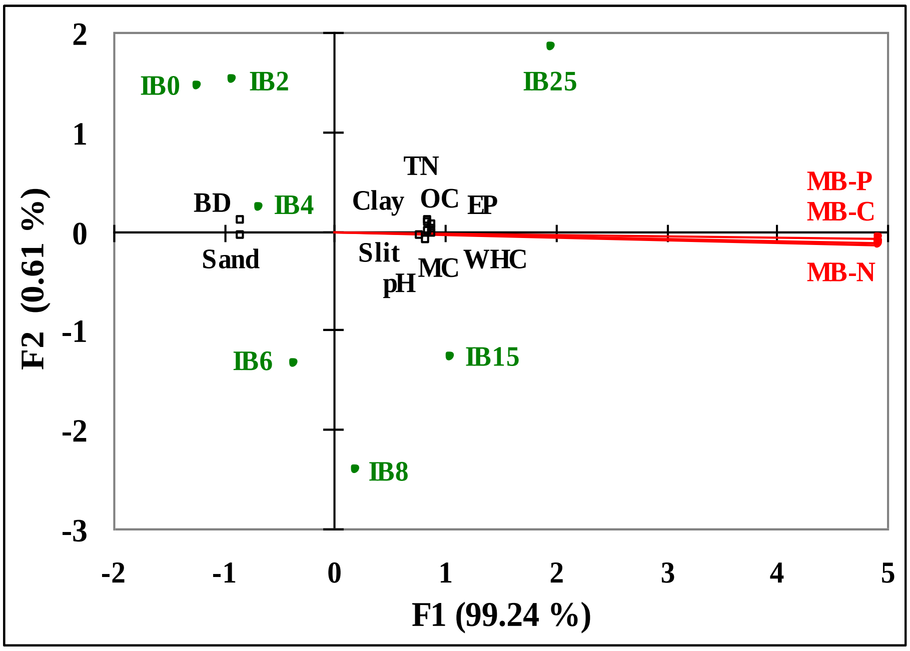

Figure 2. Redundancy analysis among seven iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) as well as nearby NF soil revealed the highest absolute scores on each of the first two axes of biological interest with site codes.

CONCLUSION

Assessment of microbial biomass pool is important, because of the microbial associations with organic matter and nutrient cycling critical for establishment of sustainable ecosystem. Microbial biomass responded quickly to functional changes by utilizing readily available nutrients during the early stage of mine spoil genesis. The present study was performed to evaluate the degree of variability in physico-chemical properties and microbial biomass C, N and P among mine overburden spoil ($IB_0 \rightarrow IB_{25}$) and NF soil. Gradual improvement in organic C, total N and extractable P was observed due to vegetation development, increased in clay%, and microbial mineralization, which provide soil structural stability over time. Assessment of microbial biomass pool indicated gradual improvement, and hence considered as biomarkers for monitoring mine spoil genesis supporting the sign of restoration. The MB-C:OC ratio is reported to be reliable microbiological index for evaluating the status of mine spoil genesis in restored ecosystem. Stepwise multiple regression analysis can able to quantify the contribution of physico-chemical indices influencing the degree of variability in microbial biomass pool across the sites. Principle component analysis can able to segregate seven different iron mine overburden spoil in chronosequence and NF soil into independent clusters. Further, redundancy analysis revealed the variation in microbial biomass pool in response to different physicochemical properties, which can be used as biomarkers for the assessment of mine spoil genesis in chronosequence iron mine overburden spoil over time.

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