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Research article

Microbial population dynamics influence basal soil respiration and it's impact on mine spoil genesis in chronosequence iron mine overburden spoil

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Abstract

Assessment of microbial biomass pool and microbial respiration have been used as sensitive soil quality indicators of mine spoil genesis and served as useful criteria for successful rehabilitation of ecologically disturbed mining areas. The variation in moisture, organic C and microbial biomass C in different age series iron mine overburden spoil and nearby NF soil were analyzed. The analysis showed consistent increase in moisture (6.643-11.329)%, organic C (0.142-2.228)% and microbial biomass C (51.324-648.719) µg/g spoil from IB_0 to IB_{25} with increase in age of iron mine overburden spoil. Gradual improvement in organic C was found to be correlated with moisture ($r = 0.996$; $p < 0.01$) and microbial biomass C (0.999; $p < 0.01$). Besides, MBC:OC and BSR:OC ratio can be used as sensitive indicators of mine spoil reclamation, which were found to be relatively higher in IB_0 compared to NF soil. Wide variation in microbial community composition, basal soil respiration (0.158-0.463) µg CO_2-C/g spoil/hr and microbial metabolic quotients was observed across the sites over time. Stepwise multiple regression analysis was performed to quantify the contribution of microbial communities, moisture, organic C, microbial biomass C explaining the variability in microbial soil respiration. Principal component analysis was able to discriminate seven iron mine overburden spoil and NF soil into independent clusters, which correlated well with mine spoil genesis and reclamation progress. Redundancy analysis illustrated the contribution of moisture content, organic C, microbial biomass C and microbial community dynamics towards the shift in basal soil respiration useful for monitoring mine spoil restoration.

Key words: iron mine spoil, organic C, microbial community, basal soil respiration.

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1. Introduction

Soil quality assessment acquires an important dimension concern to the long-term strategies implemented for soil conservation, health and ecosystem sustainability, which not only rely on the climatic conditions, soil characteristics,

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vegetational patterns, anthropogenic influences, but also the interactions among them. The variation in soil quality among different age series iron mine spoil in chronosequence can be estimated using several biological indicators associated with physico-chemical characteristics and biological activities such as microbial biomass and basal soil respiration. Microbes are very sensitive to environmental perturbations that alter microbial community composition and activities that provide early signs of anthropogenic disturbances and predict the progress of reclamation. The abundance and distribution of different microbial populations is influenced by soil physico-chemical and biological properties [1], land uses [2], elemental composition and available nutrients [3], which determine the microbial community composition by facilitating habitats and nutrient resources for their growth and proliferation [2]. Microbial community composition and associated activities reflect microbial metabolic response as the measure of reclamation progress in different age series iron mine overburden spoil over time.

Basal soil respiration is considered as sensitive biological variable for perturbation studies and biomass determination, which represent overall microbial activity reflecting the intensity of decomposition and availability of slow flowing carbon through mineralization for microbial maintenance [4-7]. Higher microbial respiration indicates intense nutrient cycling mediated by relatively higher microbial population with consequent higher consumption of energy [8,9]. Therefore, the rate of microbial respiration is considered as the potential measure of microbial turnover rate in soil [10] and used to examine microbial activity, which is considered as the key factor influencing ecosystem functioning and soil management [11].

Besides, microbial basal soil respiration supplements positive relationship with soil development, but decreases with ecological succession. Microbial metabolic quotient (qCO_2) was determined to assess the degree of soil biological perturbance, which expresses the CO_2-C evolved per unit of microbial biomass C and time [12]. Relatively higher microbial metabolic quotient indicates more CO_2-C loss per unit microbial biomass for its maintenance. Conversely, lower value of microbial metabolic quotient indicates relatively less microbial maintenance and more carbon assimilation [13]. Soil disturbances causes decrease in microbial efficiency and increase the microbial metabolic quotient (qCO_2), because microbes requires more energy to maintain biological equilibrium and predict the progress of mine spoil reclamation. Therefore, the assessment of microbial community composition and their activity is pre-requisite for ecological restoration studies facilitating mine spoil genesis over time.

Soil microbes play pivotal roles in nutrient cycling, soil structure and soil fertility [14-17]. However, microbes are sensitive to environmental perturbations that influence their community composition and activity. Microbial activity can be monitored through the assessment of basal soil respiration in chronosequence iron mine overburden spoil. Several investigations have suggested the relationship between microbial activity, basal soil respiration, microbial biomass C and organic C [18-20]. Besides, the variables that influence the shift in basal soil respiration among different soil profiles include physico-chemical properties, nutrient availability, temperature, pH, presence of heavy metals and toxic contaminants [21-23]. Soil texture (*i.e.* clay percentage) had no significant effect on CO_2 production rate,

because oxygen is limited when the soil pores are filled with water interfering with the ability of microbes to respire [24]. Basal soil respiration rate is limited in sandy soil due to low organic matter and hydrological regimes, which indicate an unstable soil system. Microbial community composition can be determined by substrate availability rather than the pool size of microbial biomass C under favourable temperature and moisture [25]. Microbial basal soil respiration rate depends on bulk organic C pool and useful to evaluate microbial biomass C in combination with organic C, which provide insight into the quantum of overall soil degradation and development [26]. The variation in microbial biomass pool lead to alternation in microbial respiration rate [26,27]. Therefore, the periodic assessment of different microbiological and biochemical properties can be used to monitor soil sustainability and restoration of degraded soil [6,28,29].

The variation in microbial community composition and activity in chronosequence iron mine overburden spoil over time has considerable biological significance, which pave the way for greater understanding in the direction of improving soil quality through mine spoil reclamation. Keeping in view, the present study was designed to estimate the relative abundance and distribution of different microbial populations (azotobacter, arthrobacter, rhizobia, heterotrophic aerobic bacteria, sulfate reducing bacteria, actinomycetes, yeast and fungi) in different age series iron mine overburden spoil in chronosequence over time. There are few studies that quantitatively link microbial community structure and soil processes. Therefore, emphasis has been given to evaluate the potential impact of microbial community composition, moisture, organic C and microbial biomass C as predictors of basal soil respiration in order to link microbial community composition quantitatively with mine spoil genesis in chronosequence iron mine overburden spoil leading to reclamation progress.

2. Materials and Methods

Study site

The present study was carried out in Thakurani iron mining area of Noamundi (geographical location: 85° 28' 02.61" east longitude and 22° 8' 33.93" north latitude) maintained by M/s. Sri Padam Kumar Jain sponge mines Private Ltd. located in the revenue district of West Singhbhum, Jharkhand, India. The study site is surrounded by number of new, old and abandoned iron mine overburden, which are classified according to 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}) respectively within the peripheral distance of 10 Km from the core mining area. Besides, the nearby forest soil (NF) with well defined environmental condition adjacent to the core iron mining area was selected for comparison. The district experiences semi-arid climate with annual average rainfall estimated to be 1250.43 mm. Mean annual temperature and humidity is around 19.67°C and 20% respectively. The site is situated about 581m altitude away from the mean sea level.

Mine spoil sampling

Sampling was done in accordance with soil microbiological study [30]. During sampling, each mine overburden was divided into 3 blocks and five mine spoil samples were collected from each block randomly from (0-15) cm soil depth by digging pits of (15×15×15) cm^3 size. Mine spoil samples collected from each block were referred as 'sub-samples' and were

thoroughly mixed to form one 'composite sample'. Similar strategies were followed to obtain three composite samples from different age series iron mine overburden in chronosequence as well as nearby forest. Mine spoil samples were sieved (0.2mm mesh) and stored at 4ºC until analyzed.

Moisture content

Moisture content in different age series iron mine overburden spoil in chronosequence was determined [31] by taking 10g of mine spoil sample (W_1) and oven dried at 105^0C for 24hr till a constant dry weight (W_2) was obtained. Soil moisture (%) in mine spoil samples was calculated as: $[(W_1\text{-}W_2)/10] \times 100$.

Organic carbon

Organic carbon (OC) content in different age series iron mine overburden spoil in chronosequence was determined by titration method [32]. Oven dried mine spoil sample of 5g was taken in 500ml Erlenmeyer flask. About 10ml of 1N $K_2Cr_2O_7$ and 20ml of concentrated H_2SO_4 were added, mixed thoroughly and allowed to stand for 30 min. Thereafter, it was diluted with 200ml of distilled water followed by addition of 10ml of 85% H_3PO_4 and 1ml of diphenylamine indicator. The mixture was titrated against 1N ferrous ammonium sulphate until the colour flashed to green. Organic carbon content (%) was calculated as: $[(V_1 - V_2)/W] \times 0.003 \times 100$; where, V_1 = vol. of 1N $K_2Cr_2O_7$; V_2 = vol. of 1N $(NH_4)_2Fe(SO_4)_2.6H_2O$; W = wt. of sample (g).

Microbial biomass C

Mine spoil samples collected from different age series iron mine overburdens were stored at $(28 \pm 2)^0$C to stabilize respiration and subsequently used for the assessment of microbial biomass C (MBC). The MBC was determined by fumigation extraction method [33] through back titration with 0.04N $(NH_4)_2Fe(SO_4)_2.6H_2O$ using ferroin indicator with K_{EC} (calibration factor) = 0.38 and expressed on oven dry weight basis.

Microbial basal soil respiration

Microbial basal soil respiration (BSR) was determined by alkali absorption technique [34,35]. About 1ml of 1% glucose followed by 1ml $(NH_4)_2SO_4$ was added to 10g moist mine spoil sample. Then, 5ml of 0.05N NaOH was added to trap CO_2. For blank, the same procedure was followed without spoil sample. The samples and blank were incubated at 28°C for 24hr and titrated against 1N HCl in presence of $BaCl_2$ and phenolphthalein indicator. Microbial basal soil respiration (µg CO_2-C g^{-1} oven dry soil h^{-1}) at 28°C was calculated as: $[\{(V_o - V) \times S \times 22 \times 1000 \times 12\}/ (M \times \text{dry wt. of sample} \times t \times 44)]$; where V_o and V = volume of HCl consumed during titration of blank and sample respectively; S = strength of HCl (normality); t = incubation time; M = wt. of sample and 22 = equivalent wt. of CO_2.

Microbial metabolic quotient

Microbial metabolic quotient (CO_2-C/g microbial carbon/hr) is defined as the amount of CO_2-C respired per unit microbial biomass C per unit time, which was calculated from the mean value of MBC and BSR in different age series iron mine overburden spoil in chronosequence as well as nearby NF soil across the sites.

Enumeration of microbes

Microbial community composition in different age series iron mine overburden spoil in chronosequence and nearby NF soil was determined using selective media by spread plate technique. Azotobacter

population (AZB) was enumerated using azotobacter mannitol agar and incubated at (25-30)^{0}C for 48hr [36]. Arthrobacter population (ARB) was enumerated using Arthrobacter selective media [37]. Rhizobial count (RZB) was determined using yeast extract mannitol agar with Congo red dye to distinguish them from other bacteria [38]. Total heterotrophic aerobic bacteria (HAB) were enumerated using nutrient agar [39]. Sulfur reducing bacterial population (SRB) was enumerated using sulphate reducing medium (HiMedia) [40]. Actinomycetes (ACT) count was estimated using starch-casein agar [41] with streptomycin (40 µl/ml) and griseofulvin (50 µl/ml) to prevent bacterial and fungal contaminants [42]. Yeast counts (YES) were estimated using potato sucrose agar [43]. Besides, the fungal count (FUN) was determined using Rose Bengal agar supplemented with 50 µl/ml streptomycin [44].

Statistical analysis

Simple correlation analysis was performed to test the level of significance between moisture content, organic C, microbial biomass C, basal soil respiration and microbial communities in different age series iron mine overburden spoil using SPSS 17.0. Stepwise multiple regression analysis was performed to quantify the contribution of different microbial communities influencing basal soil respiration using Minitab 16. Principal component analysis was performed using Statistrix PC DOS Version 2.0 (NH Analytical software). Redundancy analysis was performed using XL-STAT (Version 2014.5.03).

3. Result and Discussion

Moisture content

The moisture content exhibited an increasing trend from IB_0 (6.643%) to IB_{25} (10.886%) over time across the sites (Table 1). Moisture content in NF soil (11.329%) was found to be relatively higher compared to different age series iron mine overburden spoil (Table 1). The progressive improvement in moisture content from IB_0 to IB_{25} may be due to gradual vegetation development and canopy shading that led to reduction in water loss and contribute higher moisture content with the increase in age of iron mine overburden spoil [45]. Relatively lower moisture content in IB_0 may be due to lower organic C level and exposed surface mine spoil that promote drying [46-48].

Table 1. Comparative assessments of moisture content, organic C, microbial biomass C and basal soil respiration in different age series iron mine overburden spoil and NF soil.

Soil profiles	Moisture (%)	Organic C (%)	MBC (µg/g spoil)	Basal soil respiration (µg CO_2-C/g spoil/hr)
IB_0	6.643 ± 0.206	0.142 ± 0.029	51.324 ± 3.641	0.158 ± 0.009
IB_2	6.985 ± 0.211	0.218 ± 0.024	72.943 ± 6.084	0.224 ± 0.011
IB_4	7.106 ± 0.198	0.284 ± 0.028	91.657 ± 6.358	0.256 ± 0.012
IB_6	7.422 ± 0.201	0.355 ± 0.034	111.758 ± 8.647	0.278 ± 0.015
IB_8	8.391 ± 0.168	0.815 ± 0.039	248.977 ± 12.109	0.293 ± 0.016
IB_{15}	9.915 ± 0.176	1.648 ± 0.041	472.489 ± 11.588	0.355 ± 0.021
IB_{25}	10.886 ± 0.155	2.228 ± 0.045	593.789 ± 13.427	0.432 ± 0.027
NF	11.329 ± 0.198	2.469 ± 0.052	648.719 ± 16.522	0.463 ± 0.032

Organic carbon

Wide variability in organic C content was exhibited by different age series iron mine overburden spoil, which varied from 0.142 ± 0.029% (IB_0) to 2.228 ± 0.045% (IB_{25}) across the sites (Table 1). Gradual improvement in organic C may be due to vegetation development, organic matter accumulation and nutrient retention in mine overburden spoil over time [49,50]. Relatively higher level of organic C was estimated in IB_{15} (1.648 ± 0.041%) compared to IB_8 (0.815 ± 0.039%) and IB_6 (0.355 ± 0.034%). The increase in organic C was found to be correlated with the increase in clay fraction in ecologically disturbed land [51,52]. It is evident from the study that relatively higher level of organic C were recorded in nearby NF soil (2.469 ± 0.052%) compared to different age series iron mine overburden spoil, which may be due to the gradual accumulation of organic C contributed by litter inputs from vegetation compartment and its decomposition due course of passive or active restoration [53-55]. In contrast, minimum level of organic C in IB_0 may be due to the hostile ambience of fresh iron mine overburden spoil [56-58]. Gradual improvement in organic C with the increase in age of iron mine overburden across the sites was found to be significant ($r = 0.912$; $p < 0.001$).

Microbial biomass C

The level of microbial biomass C in different age series iron mine overburden spoil in chronosequence showed an increasing trend from 51.324 μg/g spoil (IB_0) to 593.789 μg/g spoil (IB_{25}) over time (Table 1). Minimal microbial biomass C in fresh iron mine overburden spoil may be due to the heavy metal toxicity and lower input of organic C [59]. Relatively higher microbial biomass C was estimated in nearby NF soil (648.719 μg/g spoil) as compared to different age series iron mine overburden spoil [60]. Besides, relatively lower microbial biomass C was observed in IB_8 (248.977 μg/g spoil), IB_{15} (472.489 μg/g spoil) and IB_{25} (593.789 μg/g spoil) compared to nearby NF soil, which may be due to the adsorption of toxic chemicals on organic matter leading to microbial death and thereby exhibited reduced activity [49]. The study indicated significant increase in microbial biomass C with the increase in age of iron mine overburden spoil ($r = 0.974$; $p < 0.001$). There exists a strong linkage between organic C and microbial biomass C with the former acting as the "energy currency" for the later. The magnitude of microbial biomass C fluctuation in different mine spoil profiles appeared to be dependent on the available soil nutrients [61]. It was observed that soil desiccation resulted gradual decrease in microbial biomass pool in different soil profiles [62]. The variation in microbial biomass C among different age series iron mine overburden spoil in chronosequence was found o be positively correlated with organic C ($r = 0.999$; $p < 0.01$) (Table 3), which substantiated the earlier studies [63,64]. Furthermore, the positive correlation between moisture content and microbial biomass C ($r = 0.998$, $p < 0.01$) substantiated the importance of moisture variability influencing microbial biomass pool over time (Table 3). The study indicated that the shift in microbial biomass pool was found to be closely related to moisture fluctuations in different age series iron mine overburden spoil over time.

Microbial basal soil respiration

Microbial basal soil respiration rate and its fluctuations in different age series iron mine overburden spoil over time were presented (Table 1). Basal soil respiration (BSR) is considered as reflection of the availability of slow flowing carbon for microbial

maintenance, which is used as the measure of basic turnover rate in soil [10].

Significant variation in BSR (0.158 - 0.432 µg CO_2-C/g spoil/hr) was observed across the sites with minimum in IB_0 and maximum in IB_{25}, which may be due to gradual accumulation of plant litter input [65]. Comparatively lower BSR in IB_0 indicated limited availability of organic matter to promote microbial growth [26]. In contrast, BSR in NF soil (0.463 µg CO_2-C/g soil/hr) was found to be relatively higher as compared to different age series iron mine overburden spoil, which may be due to the gradual input of organic C from the vegetational component. The variation in BSR exhibited positive correlation with microbial biomass C ($r = 0.934$; $p < 0.01$), organic C ($r = 0.935$, $p < 0.01$) and moisture content ($r = 0.952$, $p < 0.01$) across the sites (Table 3).

Integrating quotients

The ratio of microbial biomass nutrients to soil nutrients (MBC:OC) represents the quantum of soil nutrients reflected in the microbial biomass pool [66], which can be used as an integrative quotient for monitoring iron mine spoil genesis. In the present study, the MBC:OC ranged from 3.61% to 2.66% with maximum in IB_0 and minimum in IB_{25} (Table 2), which may be due to the variation in microbial community composition and their efficiency to utilize organic C as energetic substrate [67,68]. Relatively higher MBC:OC ratio in IB_0 suggested that the microbes are under stress due to metal contaminated mine spoil and are less efficient for organic C utilization [26]. Lower level of MBC:OC ratio was exhibited by NF soil (2.62%), which suggested enriched system with available soil nutrients. Comparative assessment of MBC:OC ratio in different age series iron mine overburden spoil reflect soil organic C status and can be used as biomarker for monitoring mine spoil genesis and progress of reclamation [48,56]. The estimate of microbial basal soil respiration in intact soil represents the rate of C mineralization in soil system [69]. Besides, the BSR:OC ratio revealed an increasing ranged from 1.113% to 0.194% with maximum in IB_0 and minimum in IB_{25} across the sites (Table 2). The BSR:OC ratio in the nearby NF soil was estimated to be 0.188%. The study substantiated the fact that higher value of BSR:OC ratio confirms the greater use of organic C by the existing microbial communities inhabiting in IB_0 as compared to different age series mine overburden spoil over time, which could also be ascribed to the elevated stress condition of the habitat [70].

Table 2. MBC:OC, BSR:OC and microbial metabolic quotient (qCO_2) in different age series iron mine overburden spoil and nearby NF soil across the sites.

Soil profiles	MBC/OC (%)	BSR/OC (%)	Microbial metabolic quotient (CO_2-C/g microbial-C/hr)
IB_0	3.61	1.113	3.0784×10^{-3}
IB_2	3.34	1.028	3.0708×10^{-3}
IB_4	3.22	0.901	2.7930×10^{-3}
IB_6	3.14	0.783	2.4875×10^{-3}
IB_8	3.05	0.360	1.1768×10^{-3}
IB_{15}	2.86	0.215	0.7513×10^{-3}
IB_{25}	2.66	0.194	0.7275×10^{-3}
NF	2.62	0.188	0.7137×10^{-3}

Table 3. Simple correlation coefficients analysis between different microbial population and physico-chemical properties in different age series iron mine overburden spoil.

	AZB	ARB	RZB	HAB	SRB	ACT	YES	FUN	MC	OC	MBC	BSR
MC	0.900**	0.859*	0.926**	0.959**	-0.889**	0.919**	0.944**	0.935**	1			
OC	0.869*	0.816*	0.895**	0.939**	-0.856*	0.883**	0.914**	0.904**	0.996**	1		
MBC	0.879**	0.829*	0.906**	0.947**	-0.871*	0.898**	0.928**	0.915**	0.998**	0.999**	1	
BSR	0.942**	0.904**	0.920**	0.936**	-0.922**	0.945**	0.933**	0.950**	0.952**	0.935**	0.934**	1

** Correlation is significant $p < 0.01$,*Correlation is significant $p < 0.05$ level (2 tailed test).

Microbial metabolic quotient

The amount of CO_2-C evolved for unit microbial biomass C per unit time is known as microbial metabolic quotient (qCO_2) [71], which reflects the relationship between microbial respiration with their biomass and used as an indicator for microbial mediated C mineralization. More efficient the microorganisms function the greater is the C mineralization as the ecosystem succession progress resulting in lower metabolic quotient [72]. The qCO_2 was found to be maximum in IB_0 (3.0784×10^{-3} CO_2-C/g microbial-C/hr) and minimum in IB_{25} (0.7275×10^{-3} CO_2-C/g microbial-C/hr) over time across the site (Table 2). The qCO_2 exhibited by nearby NF soil (0.7137×10^{-3} CO_2-C/g microbial-C/hr) was found to be relatively less as compared to different age series iron mine overburden spoil. Relatively higher qCO_2 in IB_0 may be due to the lack of organic C and presence of microbial community with 'r'- strategy ecotype, which are known for lower C mineralization *i.e.* they evolved more CO_2-C per unit degradable carbon substrate [26]. Minimum qCO_2 in IB_{25} spoil is may be explained on the basis of the presence of complex detritus and more efficient microbial community with 'k'- strategy ecotype [73]. In addition, more the labile C that is readily decomposable would favour opportunistic 'r'-strategy ecotype over enzyme procedures 'k'-strategy ecotype [74,75]. Therefore, 'r'-strategy ecotype represents disturbed soil habitat where as 'k'-strategy ecotypes shows the recovery of the disturbed soil habitat associated with low qCO_2 [26,65]. The consistent decline in microbial metabolic quotient from IB_0 to IB_{25} with the increase in age of iron mine overburden spoil over time strongly substantiate mine spoil genesis and reflect the progress of reclamation.

Enumeration of microbes

Comparative assessment of microbial communities in different age series iron mine overburden spoil and NF soil have been presented (Figure 1). The NF soil exhibited relatively higher microbial population compared to different age series iron mine overburden spoil. The variation in microbial population in terms of AZB (5×10^{-1} to 16×10^{-4}), ARB (11×10^{-2} to 42×10^{-3}), rhizobia (4×10^{-1} to 13×10^{-4}), HAB (22×10^{-2} to 31×10^{-6}), ACT (2×10^{-2} to 32×10^{-3}) count was observed with minimum in IB_0 and maximum in IB_{25} across the sites. However, the SRB count exhibited a reverse trend from IB_0 (41×10^{-6}) to IB_{25} (6×10^{-2}). Relatively higher yeast (3×10^{-1} to 25×10^{-2}) and fungal (4×10^{-1} to 13×10^{-3}) populations were recorded in IB_{25} compared to different age series iron mine overburden spoil. It is evident from the study that the unmined soil harbors comparatively higher microbial population, which was found to be approximately 2-4 logs greater than the rehabilitated soil [76].

Azotobacter belongs to family *Azotobacteraceae*, which is

chemoheterotrophic, obligately aerobic, dinitrogen-fixing bacteria. Relatively minimal AZB count was observed in IB_0 compared to IB_{25}, which may be due to the nutrient deficient situation in IB_0. Gradual increase in AZB population was observed with the increase in age of iron mine overburden spoil, which reflect the change in environmental conditions over time. Comparative assessment of arthrobacter count showed an increasing trend from nutrient deficient condition (IB_0) to nutrient enriched soil (NF). The variation in ARB count across the sites may be due to the variability in available soil organic matter, which is used as the principal sources of carbon and energy [37]. The rhizobial count exhibited an increasing trend from IB_0 to IB_{25}. Relatively higher RZB count was observed in NF soil compared to different age series iron mine overburden spoil because of their highly specific symbiotic association with leguminous plants to accelerate biological nitrogen fixation [66]. Besides, the heterogeneity in vegetation pattern over time may be the possible reason for such variation in rhizobial counts across the sites [77]. Further, the colonization of RZB population provides an important alternative for the recovery of iron mine overburden spoil [78]. The HAB population was found to be relatively higher than other microbial population, which may be due to their efficiency of decomposing cellulose, lignin, chitin, keratin, hydrocarbons, phenol and other substances. Besides, HAB can catalyze the dis-similatory reduction of iron and sulphur contaminants [79] and hence their population exhibited an increasing trend from IB_0 to NF soil. The HAB population in IB_4, IB_6, IB_8 and IB_{15} was found to be comparatively less than IB_{25}, which may be due to the environmental stresses brought by the contamination, which led to the reduction in microbial diversity [80].

In contrast, the SRB count showed a decline trend from IB_0 to IB_{25} (Figure 1). Being deficient in soil nutrients with enriched pyrite (FeS_2) contamination, IB_0 provides the suitable microenvironment for SRB growth and proliferation [66,81]. SRB are heterogeneous group of anaerobic microbes that use sulphate as terminal electron acceptor and subsequently use simple organic and inorganic compounds as their electron donor [82,83]. The involvement of SRB in sulphur utilization of sulphur compounds [84] and sulphate reduction accounted for organic C mineralization indicating their importance in sulphur and carbon cycles. Further, wide variation in actinomycetes count was observed in different age series iron mine overburden spoil over time. Their relative distribution and dominance in soil depends on soil physico-chemical properties such as soil moisture content, pH, aeration and nutrient content [85]. Further, ACT are relatively acid-tolerant, which makes them possible to survive even in fresh mine spoil [86]. The relative distribution of yeast is influenced by several environmental factors such as soil moisture, pH, organic C, aeration and nutrient availability, which may be the possible reason for higher YES count in IB_{25} compared to different age series iron mine overburden spoil. Comparatively less fungal population was estimated in IB_0 may be due to their high acidic condition, which substantiated the previous studies [87,88]. Higher fungal population in IB_{25} may be due to prevailing favourable soil physico-chemical properties that enhance microbial colonization [89,90], vegetation development in iron mine spoil and thereby enhance the toxic metal tolerance capacity [91]. Besides, their symbiotic relationship with the root of higher plants may be the possible reason for relatively higher fungal count in IB_{25} [92]. Several studies indicated that the shift in microbial community composition is influenced by the spatial and

temporal variability such as temperature [93], pH or salinity [94], available nutrients [95] and degree of contamination or pollutants [96].

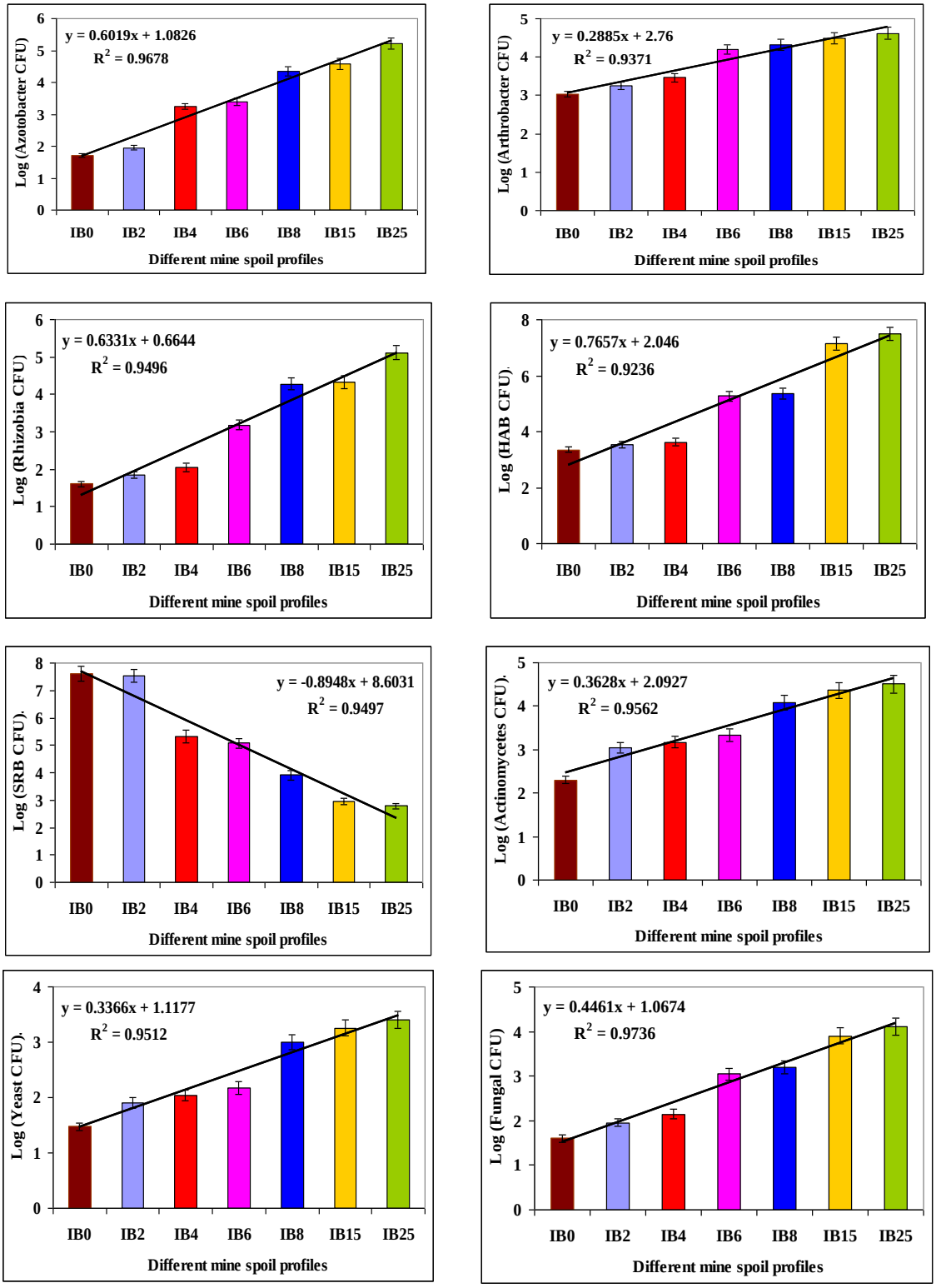


Figure 1. Comparative assessment of microbial communities in different age series iron mine overburden spoil across the sites.

Further, simple correlation coefficient analysis was performed to elucidate the relationship between microbial population and soil physico-chemical variables (Table 3). The analysis suggested that the microbial populations and BSR was estimated to be relatively higher in IB_{25} compared to different age series iron mine overburden spoil. The variation in microbial biomass C was positively correlated with the fluctuation in microbial community composition [97,98]. Soil moisture is one of the important abiotic variables influencing microbial basal soil respiration [65] regulating the physiological activity of vegetation and soil microbes [99]. The variation in mine spoil from IB_0 to IB_{25} was found to be correlated with available soil organic C, moisture content and microbial biomass C level [97,100].

Contribution of MC, OC, MBC and microbial communities

The variation in microbial basal respiration was influenced by the shift in microbial community composition, which may be due to the variability in carbon inputs across the sites [65,80]. Stepwise multiple regression analysis was performed in order to determine the contribution of different microbial communities on microbial basal soil respiration (Table 4). About 88.8% of the variability in BSR was explained by AZB as 1st variable. Additionally, 5.6% of the variability in microbial BSR was explained by MC as 2nd variable. About 81.6% of the variability in BSR was explained by ARB as 1st variable and additional 11.8% of the variability was explained by MC as 2nd variable. The 3rd variable of importance in BSR was explained by MBC. The RZB accounted 84.6% of the variability in BSR as 1st variable and additional 7% was contributed by MC as 2nd variable. Besides, the 3rd variable of importance in explaining the variability in BSR was MBC (6.9%) and a marginal effect (1.2%) by AZB as 4th variable. About 87.5% of variability in BSR was explained by HAB as 1st variable. Moreover, the SRB explained 84.9% of the variability in BSR as 1st variable and an additional 8.4% was explained by MC as 2nd variable. Further, 89.2%, 87.1% and 90.3% of the variability in BSR was explained by ACT, YES and FUN independently.

Table 4. Stepwise multiple regression analysis using microbial community dynamics as dependent variables influencing the variability in microbial basal soil respiration rate across the sites.

Parameters	Equations	R^2
Microbial basal soil respiration	= 0.06416 + 0.063 AZB	0.888
	= -0.06460 + 0.030 AZB + 0.030 MC	0.944
	= -0.2028 + 0.125 ARB	0.816
	= -0.1916 + 0.045 ARB + 0.037 MC	0.934
	= -2.1328 – 0.040 ARB + 0.383 MC – 0.0024 MBC	0.973
	= 0.09908 + 0.058 RZB	0.846
	= -0.08321 + 0.017 RZB + 0.038 MC	0.916
	= -2.47812 – 0.035 RZB + 0.426 MC – 0.00260 MBC	0.985
	= -2.18321 – 0.050 RZB + 0.375 MC – 0.00228 MBC + 0.0265 AZB	0.997
	= 0.03851 + 0.0483 HAB	0.875
	= 0.49245 – 0.0413 SRB	0.849
	= 0.08334 – 0.0161 SRB + 0.034 MC	0.933
	= -0.08589 + 0.105 ACT	0.892
	= 0.01124 + 0.111 YES	0.871
	= 0. 03873 + 0.086 FUN	0.903

The stepwise multiple regression analysis using MC, OC and microbial biomass C as dependent variables influencing microbial basal soil respiration is summarized (Table 5). About 90.6% of the variability in BSR was explained by MC as 1st variable. Additionally, 6.1% of the variability in BSR was explained by MBC as 2nd variable. Besides, the 3rd variable of importance in explaining the variability in BSR was RZB (1.8%) and a marginal effect (1.2%) by AZB as 4th variable. However, about 87.4% of the variability in BSR was explained by OC as 1st variable and an additional of 6.9% of the variability was contributed by AZB as 2nd variable. The MBC accounted 87.2% of the variability in BSR as 1st variable and 9.5% of the variability was explained by MC as 2nd variable. Further, the 3rd and 4th variable of importance in explaining the variability in BSR were RZB (1.8%) and marginal effect by AZB (1.2%) respectively.

Further, the principal component analysis was performed to discriminate different age series iron mine overburden spoil in chronosequence as well as nearby NF soil based on the variability in moisture, organic C, microbial biomass C, basal soil respiration and microbial communities [101]. Principal component analysis indicated that the Z1 and Z2 components explained maximum variance and their cumulative percentage of variance was 99%, which can able to segregate seven different age series iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) spoil and NF soil into independent clusters (Figure 2).

Redundancy analysis was performed in order to explain the relationship between different soil variables altogether and quantify their contribution towards the shift in moisture content, organic C, microbial biomass C and basal soil respiration. The different age series iron mine overburden spoil ($IB_0 \rightarrow IB_{25}$) and microbial community arrows including moisture content, organic C, microbial biomass C and basal soil respiration for RDA ordination were shown (Figure 3).

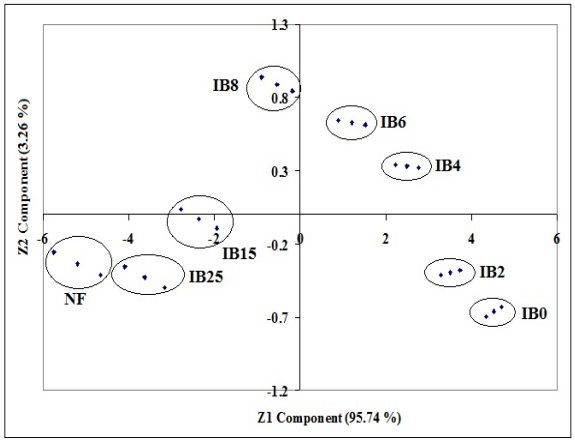


Figure 2. Principal component analysis based on soil physicochemical properties and microbial population in different age series iron mine overburden spoil.

Table 5. Stepwise multiple regression analysis using organic C, microbial biomass C and moisture as dependent variables influencing the variability in microbial basal soil respiration rate across the sites.

Parameters	Equations	R^2
Microbial basal soil Respiration	= -0.1403 + 0.0519 MC	0.906
	= -1.4848 + 0.2612 MC – 0.00158 MBC	0.967
	= -2.4781 + 0.4256 MC – 0.00260 MBC – 0.035 RZB	0.985
	= -2.1832 + 0.3748 MC – 0.00228 MBC – 0.050 RZB + 0.0265 AZB	0.997
	= 0.2024 + 0.102 OC	0.874
	= 0.1189 + 0.052 OC + 0.036 AZB	0.943
	= 0.1952 + 0.00038 MBC	0.872
	= -1.4848 – 0.00158 MBC + 0.261 MC	0.967
	= -2.4781 – 0.00260 MBC + 0.426 MC – 0.035 RZB	0.985

About 99.48% of the variability was explained based on the datasets through the canonical sum of the eigen values. The AZB, ARB, RZB, HAB, ACT, YES and FUN exhibited increasing trend in the direction of IB_6, IB_8, IB_{15} and IB_{25} supporting relatively higher moisture content and organic C leading to higher microbial biomass C and basal soil respiration, while SRB increased towards IB_0, and IB_2 suggesting a degraded land enriched with pyrite contaminants (Figure 3). The study clearly suggested that the shift in moisture content, organic C, microbial biomass C and basal soil respiration may occur in response to altered microbial community composition that affect microenvironment with possible impacts on available soil nutrients. The analysis provided an insight into the multifaceted nature of the microbial community structure that influence the basal soil respiration in chronosequence iron mine overburden spoil over time [58,102].

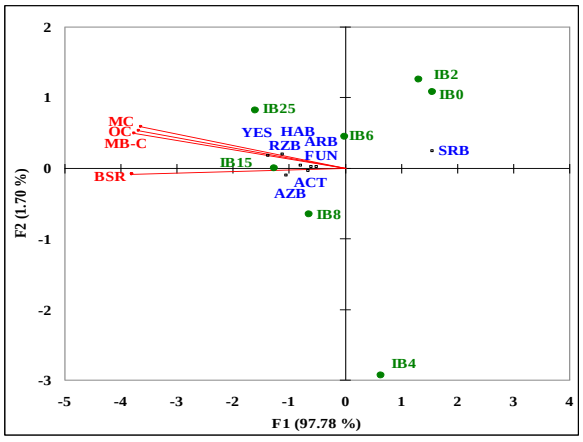


Figure 3. Redundancy analysis based on physico-chemical properties and different microbial population in chronosequence iron mine over burden spoil.

Conclusion

The variation in physicochemical properties in chronosequence iron mine overburden spoil is due to the shift in microbial community composition over time. The anthropogenic activities lead to stress condition of fresh iron mine overburden spoil. However, the gradual accumulation of organic C and increase in moisture shift the microbial community structure and alter microbial biomass pool across the sites over time leading to an increase in microbial basal respiration rate. The study indicated that the microbes respond quickly to the changes occurring in disturbed sites based on the availability of nutrient pool reflecting the progress of mine spoil genesis. Thus, the assessment of microbial basal soil respiration can be used as a sensitive indicator for monitoring mine spoil genesis and perturbation studies due to the contribution of microbial biomass pool towards the nutrient flow, organic matter turnover and structural stability reflecting the pace and progress of mine spoil reclamation.

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