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Contribution of Organic Carbon, Moisture Content, Microbial Biomass-Carbon, and Basal Soil Respiration Affecting Microbial Population in Chronosequence Manganese Mine Spoil

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ABSTRACT

The research was carried out to determine the potential effect of microbiota, organic carbon, percentage of moisture content, and microbial biomass concentration as an evaluator of variation in basal soil respiration rate. Relative distribution and composition of the microbial population were estimated from six different chronosequence manganese mine spoil (MBO0, MBO2, MBO4, MBO6, MBO8, MBO10) and forest soil (FS). The variation was seen in moisture content (6.494±0.210-11.535±0.072)%, organic carbon (0.126±0.001- 3.469± 0.099)%, MB-C (5.519±1.371- 646.969± 11.428) $\mu g.g^{-1}$ of soil. A positive correlation was shown between OC with MB-C (r = 0.938; p< 0.01) and moisture content (MC) (r = 0.962; p< 0.01). Variation in the basal soil respiration (BSR) and microbial metabolic quotients (MMQ) was shown to range between 0.352 ± 0.007- 0.958 ±0.014µg CO_2-$C.g^{-1}$ and 6.5×10^{-3} - 1.481×10^{-3} µg CO_2-$C.g^{-1}$ microbial-$C.h^{-1}$ with BSR: OC from (2.793-0.276)% respectively. This result shows that there is a gradual increase in OC, MC, MB-C, and BSR across seven different sites due to progressive enhancement in soil fertility that leads to the initialization of succession. Stepwise multiple regression analysis further confirms the degree of variability added by microbial biomass C, moisture content, organic carbon, and microbial population on basal soil respiration in microbes. Principal component analysis enables the differentiation of seven different soil profiles into independent clusters based on cumulative variance given by physico-chemical and microbial attributes that indicate the level of degradation of land and act as an index to restore soil fertility.

INTRODUCTION

The soil is a composite, highly porous, and dynamic form of earth components that bears metabolically active microorganisms from major kingdoms of life. In the current decades, the diversified microbiota of soil plays an essential role in many biological interactions that may be crucial for the cycling of organic carbon, nitrogen, and phosphorus and introduced soil as a multifunctional system that does lots of functions at the same time (Kopittke et al. 2022, Evangelista et al. 2023) Emancipation and assimilation of nutrients from biomass is readily absorbed by growing plants (Sivaranjani & Panwar 2023) and in this regards the microbial biomass reflects as both source and sink of nutrients (Smith et al.1986).

Respiration in the ecosystem provides energy that is primarily contributed by different functional elements of the ecosystem. Soil carbon is released to the atmosphere through respiration by activities of heterotrophic organisms present in the soil that regulates CO_2 level in the environment (Sáez-Sandino et al. 2023). Besides, microbial respiration is the ultimate measure of microbial activity, and its contribution to the process of soil genesis in degraded land generally complies with the determination of microbial biomass but is somehow more variable. Respiration in terms of microbial biomass (i.e., respiration occurs per unit microbial biomass) commonly decreases with respect to increasing microbial biomass (Helingerova et al. 2010), as has been observed from overburdened mine spoil along with all soil types (Santruckova & Straskraba 1991). Thus, with increasing microbial biomass, respiration might stagnate (Helingerova et al. 2010). Microbial respiration can be directly measured by conducting field experiments in soil or from soil taken into the lab for in-vitro analysis. The respiration at the ecosystem level must depend on both environmental and biological components such as structural constituents, microbial action in soil, qualitative and quantitative measurement of organic carbon, available moisture content, and nutrients (Amundson 2001). Besides, MB-C and basal soil respiration are also used to analyze the changes in microbial community

distribution and action among different overburdened mine spoil.

The areas after mining activities serve as an admissible opportunity to explore the study of soil microbial community development during the processes of pedogenesis. Microorganisms first colonized excavated soil and these microbes undergo significant changes in successional pattern during the process of soil formation. Microbial community configurations in soil play a primary role in the organization of nutrient cycles as well as the transfiguration of organic matter and regulate soil habitat by catalyzing numerous biochemical and biophysical reactions (Philippot et al. 2024). Physico-chemical and biological attributes of soil are affected by the activity and interaction of microorganisms (Tangjang & Arunachalam 2009), and community dynamics of microbes are also transformed by land utilization patterns and the availability of minerals in soil that measure the existence of microbial community by providing nutrients for growth and habitats to live (Kourtev et al. 2003). Further, the accessibility of microbes in different overburden spoil is largely influenced by variations in the concentration of organic carbon, which has a direct correlation with nitrogen cycling (Griffiths et al. 2012).

The rate of respiration in different microbial populations has been measured in various ecosystems to assess microbial interactions in soil, nutrient turnover and cycling of carbon and another organic solute. This metabolic process is also helpful in analyzing the soil contaminated with heavy metals because microbes are highly sensitive to heavy metal concentrations above critical levels (Kubat et al.1999). Besides, the factors that possess a major impact on respiration include physical-chemical and biochemical ease of access to the substrate by microbes (Davidson et al. 2006), availability of water and diffusion of a substrate (Davidson & Janssens 2006), dynamics of microbial communities (Monson et al.2006). Additionally, soil moisture is recognized as one of the efficacious environmental factors regulating soil respiration (Xu & Qi 2001).

As responsive indicators of the restoration process, mine spoil genesis converges on the gradual shifting of the composition of microbial population and inter and intra-specific relationships (Harris 2003). Though the restoration of mine spoil is a relatively longer process, it is necessary to traverse the shifting of microbial community distribution using highly responsive soil quality biomarkers.

Thus, a comprehensive study of microbial respiration rates in various mine spoil and the factors majorly affecting the respiration rate in microbes give further knowledge about the understanding of microbial distribution. Based on the above information, the current investigation was formulated to examine the relative abundance, composition, and action of microbial communities in different manganese overburden spoil collected in a chronosequence manner (fresh mine spoil: FMS; 2-year dump: MBO2; 4-year dump: MBO 4; 6-year dump: MBO 6; 8-year dump: MBO8; 10-year dump: MBO10; Forest soil: FS) across the sites.

MATERIALS AND METHODS

Study Site

The study was carried out at Kanther Manganese mines, Koira, Sundargarh, Odisha (geographical location: between 85A°20'09.67" east longitude and 21°53'45.34" north latitude) maintained by Rangta mine PVT Ltd. Out of the total geographical area about 73,653 ha area 14,796 ha are the mineralized area of Koira.

Sampling

Sampling was done by following the soil microbiological methods of Parkinson et al. 1971. For sampling, each over-dump site was divided into 5 blocks, and five soil samples were collected randomly from 0-15 cm soil depth by spading pits of (15 x 15 x 15) cm^3 size in each block and referred to as sub-samples, forming composite sample by mixing thoroughly. These samples were obtained from each site in January for analysis and subjected to sieving (2 mm mesh size) for further characterization.

Moisture Content (MC)

The moisture content from seven different soil profiles was estimated by the methods proposed by Mishra 1968. A freshly collected soil sample of about 10g (W1) was taken and kept oven-dried at 105°C for 24 h up to a constant dry weight (W2) was achieved.

Soil moisture (%) = [(W1- W2)/10] x 100

Organic Carbon Content (OC)

A partial oxidation method was used to determine soil organic carbon (Walkly & Black 1934).

Microbial Biomass-C (MB-C)

For stability of respiration and further analysis, freshly collected soil samples were stored at (28 ± 2)ºC. The fumigation extraction method was used to determine microbial biomass carbon from seven different soil samples by using the methods of Vance et al.(1987).

Basal Soil Respiration (BSR)

Methods of Witkamp (1966) followed by Ohya et al. (1988) are used to calculate basal soil respiration, measured by

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calculating the concentration of CO_2 released from the soil through the alkali absorption technique.

Microbial Metabolic Quotient

Microbial metabolic quotient (CO_2-C.g^{-1} microbial carbon.h^{-1}) is defined as the amount of CO_2-C respired per unit MB-C per unit time, and the calculation was done from the ratio of the mean value of MB-C and basal soil respiration (BSR) from seven different overburden spoils.

Enumeration of Microbes

Relative distribution of microbes in soil was performed using serial dilution techniques. Colonies were isolated using selective media by standard spread plate dilution technique. Relative distribution of Azotobacter populations (AZB) was analyzed using *Azotobacter* mannitol agar media and kept for incubation in 48 h incubated for 48 h at 25-30ºC (ATCC 1992). *Arthrobacter* (ARB) isolating media was used to isolate the *Arthrobacter* population (Hagedorn & Holt 1975). Yeast extract mannitol agar (Vincent 1970) was used for rhizobial count (RZB) containing Congo red dye to differentiate them from other bacteria from seven different soil samples. Sulphur-reducing bacteria (SRB) population counted by using medium (Hi-Media); (Eaton et al. 2005). Enumeration of actinomycetes populations (ACM) was done using (CSA) starch-casein agar (Hunter-Cevera & Eveleigh1990). The fungal population (FUN) was enumerated using rose Bengal agar media supplemented with streptomycin (50 µl.mL^{-1}) to inhibit bacterial contaminants (Alef & Nannipieri 1995). Yeast count (YES) was determined using potato sucrose agar [500 mL potato extract; 20 g.L^{-1} sucrose; 1mL trace metal solution; 500 mL distilled water; pH 6.7] (Krishna et al. 2001).

Statistical Analysis

To examine the test of significance among different physico-chemical parameters like OC, MC MB-C with BSR, and microbial populations from different chronosequence mine spoil, a simple correlation analysis was performed using SPSS (Version 16.0). To quantify the role and contribution of microbial populations towards variability in BSR, stepwise multiple regression analysis was performed using STATA 15X-64 software. The principal component analysis (PCA) was performed to summarize the large data tables collected from the above calculations using Past 4.06 software.

RESULTS AND DISCUSSION

Estimation of MC, OC, MB-C and BSR

In the current investigation, moisture content was estimated from seven different soil samples that resulted in an increasing pattern from fresh mine spoil (MBO 0; 6.494±0.210) to native forest soil (FS; 11.535±0.072). Organic carbon was estimated from overburden dump soil freshly collected, and the percentage of organic carbon increased from MBO (0.126±0.001) to native forest soil (3.469± 0.099). The same increasing pattern was followed in the case of an amount of microbial biomass carbon (MB-C). The rate of basal soil respiration was estimated from seven chronosequence manganese mine spoil and represented in Table 1 along with other attributes which were increasing from fresh mine spoil (MBO0,0.352 ± 0.007 µg CO_2-C.g^{-1} soil.h^{-1}) to relatively higher in ten-year dump soil (MBO10,0.921 ±0.012 µg CO_2-C.g^{-1} soil.h^{-1}) and further increasing in case of FS (0.958 ±0.014 µgCO_2-C.g^{-1} soil.h^{-1}).

Enumeration of Microbes

In the current study, enumeration of microbial communities and their relative availability was denoted in terms of colony-forming unit (CFU.g^{-1} soil) (Table 2). The variations that occur in terms of their relative abundance of microbiota among seven different chronosequence manganese mine spoil were shown in terms of $\log_{10}$ transformed of CFU.g^{-1} soil (Table 2). The current study specified the increasing pattern of *Azotobacter* count (AZB) from fresh mine spoil

Table 1: Table showing moisture content, organic carbon content, microbial biomass –C and BSR in chronosequence manganese mine spoil.

Soil types	MC %	OC%	MB-C [µg.g^{-1} soil]	BSR [µ g CO_2-C.g^{-1} soil.h^{-1}]
MBO0	6.494±0.210	0.126±0.001	5.519±1.371	0.352 ± 0.007
MBO2	7.496±0.127	0.312±0.013	124.911±2.587	0.392 ± 0.011
MBO4	7.72±0.101	0.626±0.1	244.401±5.392	0.594 ±0.016
MBO6	8.819±0.129	1.247± 0.031	431.163±8.378	0.842 ±0.013
MBO8	9.928± 0.8	1.687± 0.0431	498.359± 13.126	0.914 ±0.015
MBO10	10.72±0.18	2.048±0.083	540.942± 6.025	0.921 ±0.012
FS	11.535±0.072	3.469± 0.099	646.969± 11.428	0.958 ±0.014

± denotes the standard deviation

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Table 2: Relative distribution of microbial community and their abundance among seven different manganese mine spoil.

Microbial populations	CFU.g^{-1} dry wt. soil in (0-15) cm soil depth						
	MBO 0	MBO 2	MBO 4	MBO 6	MBO 8	MBO 10	FS
Azatobacter	12×10^{-1}	29×10^{-1}	26.0×10^{-2}	52×10^{-3}	46.2×10^{-4}	58.2×10^{-4}	61×10^{-4}
Arthrobacter	25×10^{-2}	29×10^{-2}	79×10^{-2}	10.2×10^{-3}	21.5×10^{-4}	31.2×10^{-4}	36.5×10^{-4}
Rhizobia	28×10^{-1}	39×10^{-1}	51×10^{-2}	8.6×10^{-3}	13.3×10^{-4}	17.8×10^{-4}	20.8×10^{-4}
Heterotrophic Aerobes	28×10^{-2}	58×10^{-2}	85×10^{-4}	98×10^{-6}	17.1×10^{-8}	21.5×10^{-8}	24.2×10^{-8}
Sulfur reducing bacteria	30.4×10^{-3}	34×10^{-3}	7.9×10^{-2}	66×10^{-1}	46×10^{-1}	15.6×10^{-1}	0.8×10^{-1}
Actinomycetes	6×10^{-2}	15×10^{-2}	29×10^{-2}	12.8×10^{-3}	36×10^{-3}	42×10^{-3}	55×10^{-3}
Yeast	7×10^{-1}	16×10^{-1}	10×10^{-2}	23×10^{-2}	43×10^{-2}	67×10^{-2}	85×10^{-3}
Fungi	9×10^{-1}	13×10^{-2}	19×10^{-2}	25×10^{-2}	11.5×10^{-3}	23×10^{-3}	45×10^{-3}

(FMS) (MBO 0: 12×10^{-1}) to forest soil (FS) (61×10^{-4}) (Table 2). From FMS (12×10^{-1}) *Azotobacter* colony showed an effective increment (MB06, 52×10^{-3}), and then further the pattern continued up to MBO10(58.2×10^{-4}). In the case of relative availability of *Arthrobacter* (ARB), the count increased from fresh mine spoil (MBO0, 25×10^{-2}) to native forest soil (FS, 36.5×10^{-4}) at an increasing rate. Further, the rhizobial count (RZB) followed a similar trend with significant variation from 28×10^{-1} (FMS) to 20.8×10^{-4} (FS). The population of heterotrophic aerobes (HAB) increases from FMS $28\times10^{-2}$to MBO 10(21.5×10^{-8}) at an increasing rate as compared to FS (24.2×10^{-8})**.** The highest sulfur-reducing bacterial count (SRB) was found in MBO0 (30.4×10^{-3}) and decreased towards MBO 10 (15.6×10^{-1}) and the population was found less in FS (0.8×10^{-1}). Further, actinomycetes count (AMC) varies from 6×10^{-2} (FMS) to 55×10^{-3} (FS). Relative distribution of yeast (YES) count showed progressive increment from fresh mine spoil(7×10^{-1}) to ten-year dump soil MBO 10 (67×10^{-2}) and highest at forest soil FS (85×10^{-3}). Fungal count was found to be higher in the case of ten-year dump soil MBO10 (23×10^{-3}) as compared to fresh mine spoil MBO0 (9×10^{-1}) favors a suitable environment for microbial growth and action (Table 2).

Discussion

Basal soil respiration depicts the availability of steady and slow-flowing carbon for nurturing microbial populations and determines the basic turnover rate in soil. BSR from different manganese overburden dump soil has shown significant variation from fresh mine spoil (MBO0) to natural forest soil (FS) ranging from 0.352 ± 0.007 µg CO_2-C.g^{-1} soil.h^{-1} to 0.958 ± 0.014 µg CO_2-C.g^{-1} soil.h^{-1}. The rate of basal soil respiration is slow in microbes present in fresh mine spoil, substantiates the minimum turnover rate of microbes, which may be due to low accessibility nutrients present in the soil for the growth of microbes as well as the susceptibility of mine spoil to various environmental adversities. However, the gradual investment of organic C forms the vegetation remains led to an increase in BSR across different overburden dump sites (Yuste et al. 2007). The BSR: OC ratio represents an increasing pattern with higher FMS (2.793%) and minimum FS (0.276%) across the areas (Table 3). The study actualized the fact that a high BSR: OC ratio validates the greater use of the native organic C by the existing microflora in soil colonizing fresh mine spoil as compared to other soil profiles efficiently that are ultimately responsible for increasing adversities of the habitat (Killham & Firestone 1984).

Microbial Metabolic Quotient

The ratio between microbial respiration per unit biomass per unit time primarily regulates the heterotrophic respiration is known as microbial metabolic quotient qCO_2 (Xu et al. 2017), which also refers to the ratio between the release of CO_2-C to CO_2 consumed (Insam & Domsch 1988). The microbial metabolic quotient tells about the adequacy of soil microbial communities in terms of utilization of substrate/energy (Insam & Haselwandter 1989, Insam 1990). Besides, the respiratory process is affected by microbial community composition, microbial biomass and basal soil respiration, ultimately affecting microbial metabolic quotient (Jiang et al. 2013).

Table 3: Percentage of organic C released as CO_2-C (BSR: OC) and microbial metabolic quotients (qCO_2) from seven different manganese mine spoil.

Soil profiles	BSR/OC [%]	Microbial metabolic quotients CO_2- C.g^{-1} microbial – C.h^{-1}
MBO0	2.793	6.5×10^{-3}
MBO2	1.263	3.1×10^{-3}
MBO4	0.948	2.43×10^{-3}
MBO6	0.675	1.953×10^{-3}
MBO8	0.541	1.834×10^{-3}
MBO10	0.449	1.703×10^{-3}
FS	0.276	1.481×10^{-3}

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In the current study, the microbial metabolic quotient was shown to follow a decreasing pattern across sites from fresh mine spoil to natural forest soil. In FMS, microbial metabolic quotient was found to be higher ($6.5\times10^{-}3$) as compared to the minimum in FS (1.481×10^{-3}) (Table 3). Generally, more qualitative and nutrient-rich soil responsible for the release of less CO_2-C per unit microbial biomass gives a lower metabolic quotient (Insam & Domsch 1988, Insam & Haselwandter 1989). Detritus is lacking in mine-operated soil, leading towards less decomposition and an almost small amount of organic substrate and the microbial population is anticipated to follow the ecotype of r- a strategy that acquires more CO_2-C per unit of substrate availability. Whereas in FS, due to the presence of a good amount of residue, the soil is predominated by decomposers that are expected to follow the ecotype of k strategy that is responsible for the respiration of comparatively less CO_2-Cperunit decomposed organic substrate (Lynch & Panting 1982), known as economic metabolism given by Insam 1990. Additionally, greater the inconstant C that easily disintegrates would favor more opportunistic and unstable r‟-strategy ecotype as compared to k‟-strategy ecotype (Yuste et al. 2007), which are predominately enzyme producers. This variation in qCO_2 between fresh mine spoil (MBO0) and FS can be elaborated with respect to qualitative substitution in microbial populations. Thus, the study supports the areas exposed to more stress responsible for the release of qCO_2 as compared to fertile land due to the lack of action of microorganisms efficiently.

Enumeration of Microbes

In the current study, the relative availability of *Azotobacter*, *Arthrobacter*, rhizobia, heterotrophic aerobic bacteria, actinomycetes, yeast, and fungi are increased at an increasing rate (Table 2) that rely upon the level of accessibility of quality substrate, OC content, variations in microclimatic factors and intensity of vegetation cover with heterogeneity. Azotobacter is chemoheterotrophic, a gram-negative, free-living microbe that belongs to the family Azotobacteriaceae, which is an obligate aerobic, nitrogen-fixing bacteria. In fresh mine spoil, due to deficiency of available nutrients, *Azotobacter* count was less and showed an increasing trend up to forest soil. Arthrobacter genus belongs to the family Micrococcaceae (Paul et al. 2011) and is a non-spore-forming gram-positive bacteria that functions as a valuable dinitrogen-fixing organism. Arthrobacter colony count was increasing from fresh mine spoil to forest soil and decreasing towards acidity that prevails microbial growth that resulted in a minimal count of *Arthrobacter* population in fresh mine spoil (Hagedorn & Holt 1975).

Rhizobium genus belongs to the family Rhizobiaceae and possesses a remarkable feature because of its symbiotic relationship with legumes located in a wide range of tropical and subtropical regions and enables the growth of plants in nitrogen-deficient soils. This association has a major quantitative impact on the global nitrogen cycle and is also important ecologically and agriculturally because it contributes a considerable amount of nitrogen from the atmosphere to fix essential forms like ammonia, nitrate, and organic N_2 and is important for the stability and functioning of the ecosystem (Nakade 2013) that might be the possible reason for exhibiting less RHB count in FMS as compare to highly fertile FS. Heterotrophic aerobic bacteria count (HAB) was much higher in nutrient-rich contamination-free native forest soil as compared to MBO0 because of lacking ecological balance and equilibrium due to the continuous degradation of surface soil because of mining. Microbial communities exposed to high environmental stress were less diverse, with a low network of stability as compared to microbes with less stress (Hernandez et al. 2021). However, the relative count of sulphur-reducing bacteria (SRB) showed a decreasing pattern from MBO0 to nearby native forest soil. During the surface mining process, textural properties and geomorphic features are lost in low OC content, but the availability of pyrite from FeS_2 enables the growth of SRB in degraded soil. Actinomycetes are saprophytes that have both features of bacteria and fungi widely distributed in soil and belong to the order Actinomycetales. Distribution of this non-spore-forming positive bacteria is majorly influenced by factors like temperature, OC content, moisture content, and aeration and moisture content of soil (Arifuzzaman et al. 2010) and this may be the probable reason for high actinomycetes (ACM) count in MBO as compare to FS. Yeast (YES) is an artificial group of fungi with the unicellular form of life cycle widespread in soil across the world. Yeast has an effective impact on an aggregation of soil (Bab'eva & Moawad 1973), assists in the regeneration of nutrients (Botha 2011) but also influences ground vegetation (Cloete et al. 2009) and other organisms in soil (Yurkov et al. 2012). This might be the possible reason for the highest yeast count in fresh mine spoil (MBO0) as compared to FS, which favors the microenvironment for the existence of microbes. Fungi are microscopic cells that act like natural cycling mediums and reabsorb essential nutrients from the soil. The filamentous hyphal structure of these microorganisms forms macro-aggregates in soil by binding soil particles together, improving the infiltration of water and water-holding capacity of the soil. The fungal hyphae form a network that expands the surface area of plant roots and helps in the translocation of deficient nutrients across the plants. Additionally, this might be the possible explanation

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Table 4: Table showing simple correlation coefficients of microbial population and properties of soil.

	AZB	ARB	RZB	HAB	SRB	AMC	YES	FUN	MC	OC	MBC	BSR
MC	.950**	.959**	.936**	.932**	-.893**	.939**	.958**	.910**	1			
OC	.872*	.899**	.872*	.859*	-.864*	.847*	.972**	.824*	.962**	1		
MBC	.982**	.928**	.919**	.979**	-.944**	.960**	.970**	.899**	.976**	.938**	1	
BSR	.992**	.900**	.900**	.997**	-.945**	.955**	.923**	.866*	.928**	.864*	.983**	1

** Correlation significant at > 0.01 level; * Correlation significant at > 0.05 level

for the highest fungal count in native forest soil due to the presence of a high amount of utilizable substrate, adequate OC content, favorable moisture, and pH content. Fungi populations are also being reported in some acidic soil like fresh mine spoil (Domsch et al.1980).

In soil, microbes as a whole interact with each other, and the fundamental properties of these soil habitats are heterogenic and diversified. The structural arrangement of each soil type develops its own physico-chemical properties and microbial habitats and, at the same time, gives a definite living space for inhabitants of microorganisms to take space in a particular niche. Besides, variations in characteristics of soil due to addition, loss transformation, and transfer of energy regulate the exhibition of microhabitats in soil. The variations occur between microbial population and MC, OC, MB-C, and BSR determined by simple correlation analysis (Table 4)

According to the data provided, not only variable number of microbes are inhabitants in different overburden dumps but also diversity occurs in their metabolic activities in terms of soil respiration rate. Initialization of pioneering microbial colonies and their distribution was less in fresh mine spoil as compared to native forest soil because variation occurs due capacity of soil microorganisms to adsorb onto soil aggregates containing organo-mineral supplements and not get protected against heavy metals present in effectively high concentration. Microorganisms are highly sensitive and negatively impacted due to high concentrations of heavy metals (Gogoi & Das 2024). It may be possible that microbes and toxic heavy metals bind to the same binding site by formation of complex and spatial separation between the two did not occur (Kandeler et al. 2000). Heavy metal concentration above critical limit affect metabolic functions, and functions related to carbon and nitrogen cycle and contamination status of soil affected by heavy metals (Li et al. 2020). The variations in microbial communities positively correlated to the OC content of different overburden mine spoil (Fierer et al. 2003). The large population of fungi, bacteria, and other microbes enzymes like cellulolytic, hemicellulolytic, nitrifying, and denitrifying present in soil organic carbon make it more conducive for microbial growth and also act as a source and sink of nutrients as well as maintenance of soil fertility (Khatoon et al. 2021). Respiration mostly relies on the moisture content (Silva et al. 2015). Soil with well adequate moisture can sustain more diverse microbial communities, and optimum soil moisture maintains the microflora of soil that is responsible for the transformation of organic compounds and the detoxification of toxic metals (Borowik & Wyszkowska 2016). Among these seven overburden mines, spoil microbial community composition and action solely depend on available moisture, thus showing a positive correlation.

Step-wise multiple regression analysis from seven overburdened mine spoils by taking microbial communities as a dependent variable was summarized (Table 5). Azotobacter (AZB) and actinomycetes (AMC) contribute about 98.48% and 91.25% of the variability in basal soil respiration. Additionally, AZB, as a first variable, 0.37% contributed by SRB and MC gave a marginal effect by contributing 0.26% to microbial respiration. Arthrobacter (ARB), as the first variable, contributed 81.02% independently and explained 15.71% and 2.97% as MBC as the first variable and AZB as the second variable. The marginal effect explained by MC by contributing 0.28% to the variability of BSR that denotes optimum moisture may favor a good number of microbial communities with adequate BSR (Borowik & Wyszkowska 2016). In BSR, rhizobia (RZB) explained 81.03% variability and 15.60% contribution explained by MB-C. Heterotrophic aerobic bacteria (HAB) and sulfate-reducing bacteria (SRB) added 99.48% and 89.31% variation to the BSR independently (Table 5). 85.14% variability contributed by yeast (YES) independently, with 13.07% explained by MB-C as the first variable. The marginal effect is shown as 0.03% by Fungi ((FUN). As eukaryotes, Fungi contribute 75.04% variability to BSR and play a major role in nutrient storage as well as release (Yurkov et al. 2011). 7.04% variation is explained by organic carbon (OC), with 7.93% contributed by SRB. 76.44% contribution explained by organic carbon as the first variable that favors more microbial activity and favors more microbes to colonize with 7.42% variation. To regulate the carbon cycle in the terrestrial ecosystem, microbial biomass C plays a major role and contributes 96.62% variation to BSR. Soil moisture is a primary factor in improving soil physico-chemical properties and contributes 82.20% with 16.45% variation incorporated by MB-C to make the soil more fertile for vegetation.

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Table 5: Step wise multiple regression analysis by using microbial composition as a dependent variable influencing the variability in BSR in seven different sites.

	Equations	R^2
Microbial BSR	0.021248+ 0.16159AZB	98.48
	-0.26598+0.25559AMC	91.25
	0.25921+0.13852AZB-0.4132SRB	98.85
	0.3712+0.16060AZB-0.04423SRB-0.0362MC	99.11
	-0.36109+0.2408ARB	81.02
	0.36609-0.02355ARB+0.001237MBC	96.73
	0.25144-0.0804ARB+0.0003MBC+0.1654AZB	99.70
	0.5377-0.1633ARB+0.00092MBC+0.1144AZB-0.00645MC	99.98
	-0.03026+0.1867RZB	81.03
	0.30411-0.0039RZB+0.001163MBC	96.63
	021423-0.00648RZB+0.001MBC+0.0412AMC	96.81
	0.0158+0.09907HAB	99.48
	1.4359-0.2363SRB	89.31
	0.4791-0.0393SRB+0.00097MBC	96.89
	-0.04576+0.232185YES	85.14
	0.50839-0.13036YES +0.00172MBC	98.21
	0.51667-0.1272YES+0.00175YES-0.007865 FUN	98.24
	0.05863+0.19102FUN	75.04
	0.2097+01059FUN+0.10441OC	82.08
	1.0674+0.0377FUN+0.2929OC-0.1712SRB	90.91
	0.44851+0.19268OC	74.66
	0.206971+0.1044OC+0.1059FUN	82.08
	0.29577+0.00114MB-C	96.62
	-0.4642+0.1311MC	82.20
	0.85375-0.9232MC+0.00188MBC	98.65
	0.8598-0.09498MC+0.001877MBC-0.005922	98.66

*All R2 - values are significant at p< 0.001

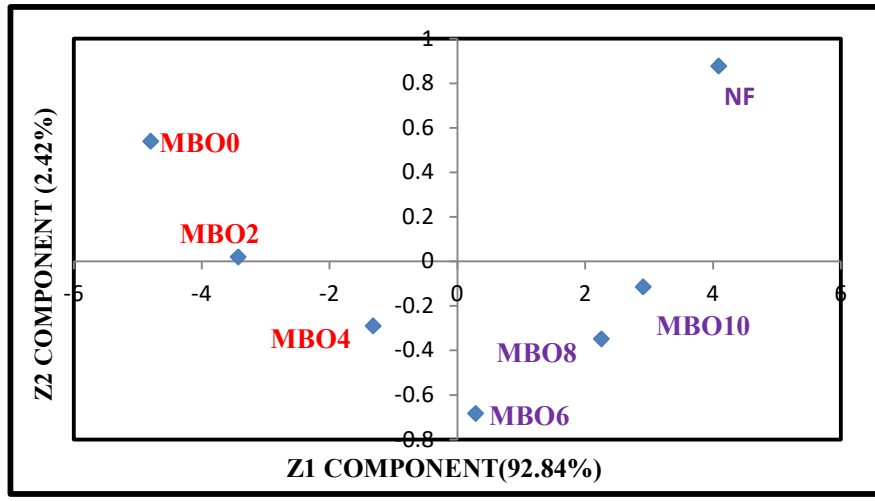

Fig. 1: PCA based on MC, OC, MB-C, basal soil respiration, and microbial population parameters in six different manganese mine spoil and nearby forests.

To acquire a clear understanding of variations among different sites based on MC, OC, MB-C, BSR, as well as microbial populations, principal component analysis was performed (Ludwig & Reynolds 1988). Principal component analysis denotes the Z1 and Z2 components that represent maximum variance with respect to soil physico-chemical and microbial parameters, contributing a maximum cumulative variance percentage of about 95.26%, which discriminates seven different soil profiles into independent clusters (Fig.1).

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CONCLUSION

The present investigation was accomplished to examine the intensity of variation among soil parameters like organic carbon, moisture content with microbial biomass carbon, microbial community distribution, and basal soil respiration in different manganese mine spoil. The variation in basal soil respiration in different sites may be due to microbial action that is greatly influenced by OC and MC of different chronosequence mine spoil. The significance of microbial flora in the functioning of the ecosystem is absorbing major attention to increasing research in microbial biomass pools that work as both a source and sink of major plant available nutrients and enhance the fertility of the soil. Thus, analysis of microbial biomass and microbial metabolic quotients signifies an index for regular surveillance of the genesis of mine spoil. The diverse results of basal soil respiration rate are due to the transformation of microbial community structure over time, and the increase in respiration rate is also encouraged by the accumulation of organic carbon and suitable moisture. The study also signifies the sudden response of microbes to the disturbed soil physico-chemical properties of overburdened mine spoil and it is shifting towards highly fertile soil with vegetation. Hence, evaluation of microbial biomass, community structure, and basal soil respiration rate used as highly responsive indicators of the genesis of mine spoil and their role in the transformation of microbe deficient infertile to microbial enriched, productive land due to their contribution towards nutrient cycling in an ecosystem, turnover of organic carbon, structural and functional stability of soil towards restoration.

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REFERENCES

Alef, K. and Nannipieri, P., 1995. *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press.

Amundson, R., 2001. The carbon budget in soils. *Annual Review of Earth and Planetary Sciences*, 29, pp.535-562.

Arifuzzaman, M., Khatun, M.R. and Rahman, H., 2010. Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. *African Journal of Biotechnology*, 9(29), pp.4615-4619.

ATCC, 1992. *Catalog of Bacteria and Bacteriophages*. American Type Culture Collection.

Bab'eva, I.P. and Moawad, H., 1973. Soil yeasts of the genus Lipomyces as soil-conditioning agents. *Eurasian Soil Science*, 8, pp.430-432.

Borowik, A. and Wyszkowska, J., 2016. Soil moisture is a factor affecting the microbiological and biochemical activity of soil.

Botha, A., 2011. The importance and ecology of yeasts in soil. *Soil Biology and Biochemistry*, 43, pp.1-8.

Cloete, K.J., Valentine, A.J., Stander, M.A., Blomerus, L.M. and Botha, A., 2009. Evidence of symbiosis between the soil yeast Cryptococcus laurentii and a sclerophyllous medicinal shrub, Agathosma betulina (Berg.) Pillans. *Microbial Ecology*, 57, pp.624-632.

Davidson, E.A. and Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedback to climate change. *Nature*, 440(7081), pp.165-173.

Davidson, E.A., Janssens, I.A. and Luo, Y., 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q10. *Global Change Biology*, 12, pp.154-164.

Domsch, K.H., Gams, W. and Anderson, T.H., 1980. *Compendium of soil fungi*. *Volume 1*. Academic Press, London.

Eaton, A.D., Clesceri, L.S. and Greenberg, A.W., 2005. *Standard Methods for the Examination of Water and Wastewater*. APHA, Washington, D.C.

Evangelista, S.J., Field, D.J., McBratney, A.B., Minasny, B., Ng, W., Padarian, J., Dobarco, M.R. and Wadoux, A.M.C., 2023. A proposal for the assessment of soil security: soil functions, soil services and threats to soil. *Soil Security*, 10, 100086.

Fierer, N., Schimel, J.P. and Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry*, 35(1), pp.167-176.

Gogoi, I. and Das, K., 2024. Heavy Metal Resistant Bacteria in Rhizospheric Soil: A Review. *Ecology Environment and Conservation*, 30, pp.S202-S205.

Griffiths, M.L., Fohlmeister, J., Drysdale, R.N., Hua, Q., Johnson, K.R., Hellstrom, J.C., Gagan, M.K. and Zhao, J.X., 2012. Hydrological control of the dead carbon fraction in a Holocene tropical speleothem. *Quaternary Geochronology*, 14, pp.81-93.

Hagedorn, C. and Holt, J.G., 1975. Differentiation of Arthrobacter soil isolates and named strains from other bacteria by reactions on dye-containing media. *Canadian Journal of Microbiology*, 21(5), pp.688-693.

Harris, J.A., 2003. Measurements of the soil microbial community for estimating the success of restoration. *European Journal of Soil Science*, 54(4), pp.801-808.

Helingerova, M., Frouz, J. and Šantrůčková, H., 2010. Microbial activity in reclaimed and unreclaimed post-mining sites near Sokolov (Czech Republic). *Ecological Engineering*, 36(6), pp.768-776.

Hernandez, D.J., David, A.S., Menges, E.S., Searcy, C.A. and Afkhami, M.E., 2021. Environmental stress destabilizes microbial networks. *The ISME Journal*, 15(6), pp.1722-1734.

Hunter-Cevera, J.C. and Eveleigh, D.E., 1990. *Actinomycetes Soil Biology Guide*. John Wiley and Sons, New York.

Insam, H., 1990. Are the soil microbial biomass and the basal respiration governed by the climatic regime? *Soil Biology and Biochemistry*, 22(4), pp.525-532.

Insam, H. and Domsch, K.H., 1988. Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites. *Microbial Ecology*, 15, pp.177-188.

Insam, H. and Haselwandter, K., 1989. Metabolic quotient of the soil microflora in relation to plant succession. *Oecologia*, 79, pp.174-178.

Jiang, Y., Sun, B., Jin, C. and Wang, F., 2013. Soil aggregate stratification of nematodes and microbial communities affects the metabolic quotient in an acid soil. *Soil Biology and Biochemistry*, 60, pp.1-9.

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Kandeler, E., Tscherko, D., Bruce, K.D., Stemmer, M., Hobbs, P.J., Bardgett, R.D. and Amelung, W., 2000. Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. *Biology and Fertility of Soils*, 32, pp.390-400.

Kopittke, P.M., Berhe, A.A., Carrillo, Y., Cavagnaro, T.R., Chen, D., Chen, Q.L., Román Dobarco, M., Dijkstra, F.A., Field, D.J., Grundy, M.J. and He, J.Z., 2022. Ensuring planetary survival: the centrality of organic carbon in balancing the multifunctional nature of soils. *Critical Reviews in Environmental Science and Technology*, 52(23), pp.4308-4324.

Khatoon, K., Anas, M., Siddiqui, Z. and Malik, A., 2021. Role of soil microbial flora in remediation of hydrocarbon stressed soils. *Microbiome and Global Climate Change*, pp.295-319.

Killham, K. and Firestone, M., 1984. Salt stress control of intracellular solutes in Streptomycetes indigenous to saline soils. *Applied and Environmental Microbiology*, 47, pp.301-306.

Kourtev, P.S., Ehrenfeld, J.G. and Häggblom, M., 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biology and Biochemistry*, 35(7), pp.895-905.

Krishna, H., Carpenter, A. and Potter, F., 2001. Effect of washing additives on the incidence of rots and an enumeration of surface microbes in stored squash. *New Zealand Plant Protection*, 54, pp.76-79.

Kubat, J., Nováková, J., Mikanová, O. and Šimon, T., 1999. Selection of microbial methods for the bioindication of soil pollution. *Pathways and Consequences of the Dissemination of Pollutants in the Biosphere II Symposium*, Praha, pp.61-75.

Li, S., Wu, J., Huo, Y., Zhao, X., and Xue, L., 2020. Profiling multiple heavy metal contamination and bacterial communities surrounding an iron tailing pond in Northwest China. *Science of the Total Environment*, 752, 141827.

Ludwig, J.A. and Reynolds, J.F., 1988. *Statistical Ecology: A Primer in Method and Computing*. John Wiley and Sons, pp. 337.

Lynch, J.M. and Panting, L.M., 1982. Effect of season, cultivation, and nitrogen fertilizer on the size of the soil microbial biomass. *Journal of the Science of Food and Agriculture*, 33, pp.249-252.

Mishra, R., 1968. *Ecology Workbook*. Oxford & IBH Publishing Co., New Delhi.

Monson, R.K., Lipson, D.L., Burns, S.P., Turnipseed, A.A., Delany, A.C., Williams, M.W. and Schmidt, S.K., 2006. Winter forest soil respiration is controlled by climate and microbial community composition. *Nature*, 439(7077), pp.711-714.

Nakade, B., 2013. Bacterial diversity in sugarcane (Saccharum officinarum) rhizosphere of saline soil. *International Research Journal of Biological Sciences*, 2(2), pp.60-64.

Ohya, H., Fujiwara, S., Komai, Y. and Yamaguchi, M., 1988. Microbial biomass and activity in urban soils contaminated with Zn and Pb. *Biology and Fertility of Soils*, 6, pp.9-13.

Parkinson, D., Gray, T.R.G. and Williams, S.T., 1971. *Methods to Study Ecology of Soil Microorganisms*. IBP Handbook No. 19, Blackwell Scientific Publications, Oxford, pp. 116.

Paul, S., Singh Rathi, M. and Prakash Tyagi, S., 2011. Interactive effect with AM fungi and Azotobacter inoculated seed on germination, plant growth, and yield in cotton (Gossypium hirsutum). *Indian Journal of Agricultural Sciences*, 81(11), pp.1041.

Philippot, L., Chenu, C., Kappler, A., Rillig, M.C. and Fierer, N., 2024. The interplay between microbial communities and soil properties. *Nature Reviews Microbiology*, 22(4), pp.226-239.

Sáez-Sandino, T., García-Palacios, P., Maestre, F.T., Plaza, C., Guirado, E., Singh, B.K., Wang, J., Cano-Díaz, C., Eisenhauer, N., Gallardo, A. and Delgado-Baquerizo, M., 2023. The soil microbiome governs the response of microbial respiration to warming across the globe. *Nature Climate Change*, 13(12), pp.1382-1387.

Santruckova, H. and Straskraba, M., 1991. On the relationship between specific respiration activity and microbial biomass in soils. *Soil Biology and Biochemistry*, 23, pp.525-532.

Silva, B.M., Santos, W.J.R.D., Oliveira, G.C.D., Lima, J.M.D., Curi, N. and Marques, J.J., 2015. Soil moisture space-time analysis to support improved crop management. *Ciência e Agrotecnologia*, 39, pp.39-47.

Sivaranjani, S. and Panwar, V.P., 2023. Soil nutrient dynamics under mountainous landscape: issues and challenges. In *Understanding Soils of Mountainous Landscapes*, pp. 131-149.

Smith, G.A., Nickels, J.S., Kerger, B.D., Davis, J.D., Collins, S.P. and White, D.C., 1986. Quantitative characterization of microbial biomass and community structure in subsurface material: a prokaryotic consortium responsive to organic contamination. *Canadian Journal of Microbiology*, 32, pp.104-111.

Tangjang, S. and Arunachalam, A., 2009. Role of traditional home garden systems in Northeast India. *Research Journal of Soil Biology*, 1, pp.1-7.

Vance, E.D., Brookes, P.C. and Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, 19, pp.703-707.

Vincent, J.M., 1970. *A Manual for the Practical Study of Root-Nodule Bacteria*. IBP Handbook of Methods No. 15. Blackwell Scientific Publications, Oxford.

Walkley, A. and Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, 37(1), pp.29-38.

Witkamp, M., 1966. Rate of CO_2 evolution from the forest floor. *Ecology*, 47, pp.492-494.

Xu, M. and Qi, Y., 2001. Spatial and seasonal variations of Q10 determined by soil respiration measurements at a Sierra Nevadan Forest. *Global Biogeochemical Cycles*, 15(3), pp.687-696.

Xu, X., Schimel, J.P., Janssens, I.A., Song, X., Song, C., Yu, G., Sinsabaugh, R.L., Tang, D., Zhang, X. and Thornton, P.E., 2017. Global pattern and controls of soil microbial metabolic quotient. *Ecological Monographs*, 87(3), pp.429-441.

Yurkov, A.M., Kemler, M. and Begerow, D., 2011. Species accumulation curves and incidence-based species richness estimators to appraise the diversity of cultivable yeasts from beech forest soils. *PLoS One*, 6(8), 23671.

Yurkov, A.M., Kemler, M. and Begerow, D., 2012. Assessment of yeast diversity in soils under different management regimes. *Fungal Ecology*, 5(1), pp.24-35.

Yuste, J.C., Baldocchi, D.D., Gershensoni, A., Goldstein, A., Misson, L. and Wong, S., 2007. Microbial soil respiration and its dependency on carbon inputs, soil temperature, and moisture. *Global Change Biology*, 13, pp.1-18.

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