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Influence of Species, Stand Age and Seasonal Dynamics on Soil Microbial Biomass Carbon in Restoring Limestone Mine

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Abstract**—** *This study evaluates the impacts of plantation species, stand age, and seasonal variation on soil microbial biomass carbon (MBC) in the restoration of degraded limestone mine soils at the Nandini Limestone Mines, Chhattisgarh, India. Four plantation species namely Dalbergia sissoo, Azadirachta indica, Tectona grandis, and Albizia procera were analyzed across three chronosequence ages (5, 15, and 25 years) and three seasonal periods (pre-monsoon, monsoon, and post-monsoon) at 15–30 cm soil depth. Soil microbial biomass carbon increased significantly with plantation age across all species, with Dalbergia sissoo demonstrating superior recovery from 62.85 ± 3.71 μg C g*$^{-1}$ *at 5 years to 99.34 ± 8.03 μg C g*$^{-1}$ *at 25 years (pre-monsoon), while monsoon peaks reached 117.64 ± 4.99 μg C g*$^{-1}$*. Seasonal patterns revealed dramatic moisture-driven increases, with monsoon MBC values (70–130 μg C g*$^{-1}$*) approximately two-fold higher than pre-monsoon values (40–110 μg C g*$^{-1}$*). Three-way ANOVA analysis revealed that plantation age (*$F_{2,72} = 137.87$*,* $p < 0.001$*), season (*$F_{2,72} = 57.67$*,* $p < 0.001$*), and species identity (*$F_{3,72} = 33.75$*,* $p < 0.001$*) all exerted significant main effects on MBC, with plantation age accounting for 47.4% of total variance, season explaining 19.8%, and species identity explaining 17.4%. All two-way and three-way interaction terms were non-significant (*$p > 0.36$*), indicating additive rather than synergistic effects. Despite substantial improvement, MBC values in 25-year plantations (mean 100 μg C g*$^{-1}$*) remained 40% depleted relative to undisturbed reference soils (157.59–170.56 μg C g*$^{-1}$*). The model explained 84.7% of total variance with minimal residual error (12.4%), demonstrating robust predictive capacity for restoration trajectories. Results demonstrate that integrating fast-growing, high-quality litter species like Dalbergia sissoo and Azadirachta indica with moisture-conserving amendments offers a promising strategy for accelerating microbial and ecosystem recovery in degraded limestone mine landscapes.*

Keywords**— *Soil microbes, soil carbon, biomass carbon, limestone mine, restoration.***

I. INTRODUCTION

Mining activities constitute one of the most significant anthropogenic disturbances to terrestrial ecosystems globally, with limestone quarrying representing a particularly intensive form of extraction that severely compromises soil structure, biological processes, and ecological functionality (Parthiban et al., 2023). India, being the world's second-largest producer of limestone with reserves exceeding 118 billion tonnes, faces substantial environmental challenges from extensive mining operations that extract approximately 350 million tonnes annually (Prathibam et al., 2023; Halder et al., 2024). These operations invariably result in topsoil removal, vegetation clearance, habitat fragmentation, and fundamental alterations to biogeochemical cycles (Sharma et al., 2000). The ecological restoration of post-mining landscapes has emerged as a global imperative, with forest-based rehabilitation strategies gaining recognition as the most effective approach for re-establishing ecosystem services and soil functionality recovery (König et al., 2023; Le et al., 2021). Forest restoration in degraded mining areas involves complex interactions between above-ground vegetation establishment, soil development, and the recovery of critical soil biological communities that drive nutrient cycling and ecosystem functioning (Witt et al., 2000; Zhang et al., 2019).

Soil microbial biomass carbon (MBC) has been established as one of the most sensitive and reliable indicators of soil health and ecosystem restoration success, representing the living component of soil organic matter that responds rapidly to environmental changes (Sharma et al., 2000; Vance et al., 1987). This parameter encompasses the total mass of living microorganisms that constitute approximately 1-4% of total soil organic carbon but exert disproportionate influence on soil processes through their roles in organic matter decomposition, nutrient release, and soil aggregation (Mummey et al., 2002; Barbhuiya et al., 2004). Unlike total organic carbon, MBC exhibits rapid responses to alterations in soil conditions, making it an invaluable early indicator for assessing restoration progress in post-mining environments (Ashraf et al., 2022). The restoration of limestone mine sites through forest plantations involves complex temporal dynamics where microbial biomass recovery is influenced by plantation age, species composition, seasonal variations, and site-specific soil conditions (Luo et al., 2020; Ngugi et al., 2020). Age-related changes in forest plantations fundamentally alter soil microbial communities through progressive organic matter accumulation, evolving root architecture, and changing litter inputs that collectively influence microbial habitat quality (Adeli et al., 2019; Pandey et al., 2007). Research has demonstrated that microbial biomass typically follows predictable successional patterns during forest development, with younger plantations exhibiting lower microbial biomass that gradually increases with stand maturity (Lladó et al., 2017).

Seasonal variations represent another critical dimension influencing soil microbial dynamics in tropical forest ecosystems, where monsoon-dominated climate patterns create distinct wet and dry periods that profoundly affect soil moisture, temperature, and microbial activity levels (Tomar & Baishya, 2020; Sarkar et al., 2024). The monsoon season typically triggers dramatic increases in microbial biomass due to enhanced soil moisture that alleviates water stress and promotes optimal conditions for microbial growth (Tomar & Baishya, 2020). Conversely, dry seasons often result in reduced microbial biomass as water limitations constrain metabolic activities (Tomar & Baishya, 2020). Tree species selection plays a pivotal role in determining restoration success, with different species exhibiting varying capacities to enhance soil microbial communities through their distinct litter quality, root exudate composition, and rhizosphere modification abilities (Shi et al., 2011; Allek et al., 2023). Fast-growing species commonly employed in tropical restoration programs, such as Dalbergia sissoo, Azadirachta indica, Tectona grandis, and Albizia procera, demonstrate markedly different influences on soil microbial development due to variations in their litter decomposition characteristics and root architecture (Li et al., 2022; Wang et al., 2011).

Despite the recognized importance of soil microbial biomass in ecosystem restoration, there remains a significant knowledge gap regarding the interactive effects of plantation age, species composition, and seasonal variations on microbial community recovery in post-mining environments, particularly in tropical regions where monsoon climates create unique restoration challenges (Thoms & Gleixner, 2013; Allek et al., 2023). The present investigation addresses this critical gap by examining the effects of different aged forest tree plantations (5, 15 and 25 years) comprising four important species on soil microbial biomass carbon dynamics across three seasonal periods in restoring Nandini limestone mine sites. This comprehensive approach enables the elucidation of temporal patterns in microbial recovery, species-specific restoration effectiveness, and seasonal modulation of soil biological processes that determine the success of forest-based restoration strategies in degraded lands.

II. MATERIALS AND METHOD

2.1 Study site

The study was carried out in Nandini Limestone Mine located in Dhamdha, District- Durg of Chhattisgarh State. The total area of mine is distributed in 1528 hectares located between Latitude N 21° 22’ 25.56’’ to N 21° 25’ 04.1” and Longitude E 81° 22’ 01.2’’ to E 81° 23’ 01.88’’ in which about 549 hectares are covered by core mining lease area and about 978 hectares are covered by outer buffer zone (Fig 1). The maximum elevation of the site is about 284 meters from mean sea level. The general ground slope is towards N, with gradient about 5°. The district receives an annual rainfall of 1130 mm in which 80% of rainfall was during the month of June-September. The study site exhibited mean annual temperature and humidity around 28.67°C and 56% respectively (Maitry et al., 2025a).

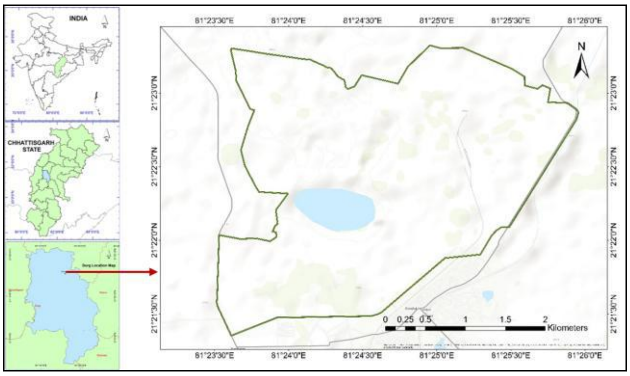

Fig 1. Geographical location of Nandini Limestone Mines (distributed over a total area of 970 hectares) in Durg, Chhattisgarh.

2.2 Selection of Sampling Plots

To assess how plantation age and species identity influence soil microbial biomass carbon during restoration of the Nandini Limestone Mine, sampling plots were established across four dominant tree species, *Dalbergia sissoo* (S1), *Azadirachta indica* (S2), *Tectona grandis* (S3) and *Albizia procera* (S4) at three stand ages (A: 5 years, B: 15 years, C: 25 years) and three seasons (Pre Monsoon, Monsoon and Post Monsoon). Each plantation type, age combination and season defined one segment, yielding thirty-six segments in total. Within each segment, rhizosphere soils were collected to capture species-, age- and season-specific microbial responses. In addition, soil from an adjacent undisturbed soil served as a reference for natural microbial biomass carbon levels.

2.3 Soil Sampling Method

Soil sampling followed a standardized chronosequence and seasonal protocol. In each of the twelve plantation segments and the reference forest, three randomly located points were marked during pre-monsoon, monsoon, and post-monsoon seasons. At each point, three blocks were established, and soil was excavated from 15–30 cm depth within a 30 cm × 30 cm × 30 cm pit. Sub-samples from each block were homogenized into a composite sample, debris and coarse fragments were removed, and the composite was divided into three replicates (Maitry et al., 2025b). All samples were passed through a 0.2 mm sieve, stored at 4 °C, and processed for microbial biomass carbon analysis within 48 hours of collection.

2.4 Analysis of Soil Microbial Biomass Carbon

Collected mine spoil samples were stored at (28 ± 2)°C for one week to stabilize respiration before estimating microbial biomass. Microbial biomass carbon (MB-C) was determined using the fumigation-extraction method (Vance et al., 1987). Two portions of moist spoil sample, each equivalent to 25 grams on an oven-dry weight basis, were prepared. One portion was fumigated with ethanol-free chloroform for 24 hours at 25°C. After fumigation, the mine spoil was extracted with 100 ml of 0.5M K_2SO_4 by horizontal shaking at 200 rpm for 30 minutes and then filtered through Whatman No. 42 filter paper. The unfumigated portion was extracted simultaneously at the start of the fumigation process. The filtered extracts were preserved at -20°C for further analysis. Organic carbon content in the filtrate was determined by wet oxidation, where 2 ml of 0.4N $K_2Cr_2O_7$ was added to 8 ml of the extract in the presence of 15 ml of an acid mixture (H_2SO_4/H_3PO_4). The mixture was placed in a 250 ml round-bottom flask fitted with a Leidig condenser and gently refluxed for 30 minutes. After cooling, the solution was diluted with 25 ml of water. The residual dichromate was measured through back titration against 0.04N $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ using ferroin as an indicator and was calculated using the following formulas:

$$\mathit{Extracted\ organic\ C}\ (\mu g/ml-1)=\frac{(H\ -\ S)\times M\times D\times E\times 1000}{C\times A}$$

Where, H = titration solution consumed by hot (refluxed) blank (in ml); S = titration solution consumed by sample (in ml); C = titration solution consumed by cold (unrefluxed) blank (in ml); M = normality of $K_2Cr_20_7$; D = volume of $K_2Cr_20_7$ (in ml); A = aliquots; E = 3 (conversion of Cr VI to Cr III).

$$\mathit{Extracted\ organic\ C}\ (\mu g/g-1\ \mathit{dry\ sample})=\frac{C\ (\mu g\ ml-1)\times K}{25\times dwt}$$

Where, K = volume of the extractant (in ml), dwt = oven dry weight of 1g of mine spoil sample (in g); 25 = weight of the sample (in g).

$$\mathit{Microbial\ biomass\ C}\ (\mu g/g-1\ \mathit{dry\ sample})\ =\frac{Ec}{Kec}=\frac{Ec}{0.38}$$

Where, Ec = Organic C extracted from fumigated sample - Organic C extracted from unfumigated sample, Kec = the calibration factor, which is equal to 0.38.

2.5 Statistical Analysis

In the present study, statistical analyses (like Mean and Standard Deviation) have been calculated using Microsoft Excel 2021.

The Duncan's multiple range test was performed using SPSS V25 software whereas Three-way ANOVA, box plot analysis and heatmap hierarchical clustering between different studied criteria were performed using Origin 2025b software.

III. RESULTS AND DISCUSSION

3.1 Species-Specific Microbial Biomass Carbon Recovery

Microbial biomass carbon (MBC) at 15–30 cm depth exhibited pronounced age-dependent increases across all plantation species (Table 1). *Dalbergia sissoo* (S1) demonstrated superior recovery, with pre-monsoon MBC rising from 62.85 ± 3.71 to 99.34 ± 6.56 µg C g^{-1} across 5–25 year plantations, and monsoon peaks reaching 117.64 ± 4.99 µg C g^{-1} (Hao et al., 2025). This superiority reflects *D. sissoo*'s robust root exudate composition rich in organic acids and amino acids (Wu et al., 2024; Srivastava et al., 2022), enabling sustained organic carbon inputs through deep rhizodeposition crucial for restoration success (Singh et al., 2025). Recent research confirms *D. sissoo* harbours specialized rhizosphere bacteria capable of producing siderophores and antagonistic compounds against soil pathogens (Srivastava et al., 2022).

Table 1. Comparative analysis of change in Soil Microbial Biomass Carbon (μgC g^{-1}) under different aged plantations at Nandani Limestone Mines and Reference Normal Soil (RNS) in different seasons at 15-30cm depth.

Age	***Species***	***Seasons***		
		Pre-Monsoon	***Monsoon***	***Post-Monsoon***
5 Years	***S1***	62.85 ± 4.539d	86.13 ± 12.004cd	77.21 ± 15.772c
	S2	58.96 ± 12.256d	82.59 ± 5.239cd	64.01 ± 6.391cd
	S3	62.41 ± 10.522c	72.06 ± 6.528c	66.79 ± 5.038d
	S4	43.53 ± 5.752c	66.50 ± 7.844c	61.25 ± 6.544c
15 Years	***S1***	81.69 ± 10.280c	101.32 ± 6.695bc	89.40 ± 6.695c
	S2	77.09 ± 7.490c	96.40 ± 6.752c	81.14 ± 12.521c
	S3	73.11 ± 10.789bc	97.75 ± 6.403b	87.16 ± 5.884c
	S4	57.48 ± 16.380bc	80.45 ± 6.806bc	68.53 ± 4.932bc
25 Years	***S1***	99.34 ± 8.027b	117.64 ± 6.114b	115.72 ± 4.979b
	S2	93.94 ± 6.302b	120.91 ± 8.455b	108.99 ± 10.354b
	S3	86.96 ± 9.510b	116.60 ± 11.161b	101.34 ± 5.392b
	S4	72.02 ± 5.810b	94.66 ±6.756b	83.41 ± 7.910b
RNS		157.59 ± 10.109a	170.56 ± 14.838a	166.31 ± 11.144a

(n=3, Average ± Standard deviation) Different letters in a row indicate the significant difference among different plantation years and RNS at $p<0.05$ according to Duncan's Multiple Range Test. Code: S1= *Dalbergia sissoo*, S2= *Azadirachta indica*, S3= *Tectona grandis*, S4= *Albizia procera*. Abbreviation: RNS= Reference Normal Soil, MB-C= Microbial Biomass Carbon.

Azadirachta indica (S2) exhibited similarly impressive patterns, with pre-monsoon MBC increasing from 58.96 ± 10.01 to 93.94 ± 5.15 µg C g^{-1} and monsoon peaks of 120.91 ± 8.46 µg C g^{-1} (Mummey et al, 2002). Its exceptional performance relates to allelopathic compounds including nimbolide B, azadirachtin, and phenolic substances that create unique rhizosphere conditions favouring beneficial microorganisms (Kato-Noguchi & Ino, 2014; Mweetwa et al., 2016). The species maintained notably higher MBC during dry pre-monsoon periods, suggesting effective buffering of microbial communities against moisture stress through diverse root exudate profiles containing alkaloids, flavonoids, and terpenoids (Wu et al., 2024; Mweetwa et al., 2016).

Tectona grandis (S3) showed moderate enhancement with pre-monsoon MBC increasing from 62.41 ± 8.59 to 86.96 ± 7.77 µg C g^{-1} and monsoon peaks of 116.60 ± 9.11 µg C g^{-1} (Mummey et al, 2002), indicating $p<0.05$ according to DMRT. However, smaller seasonal increases reflect elevated lignin content (35.7–48.0 g kg^{-1}) and high polyphenolic compounds that slow microbial decomposition (Cavalcante et al., 2020). The species' half-life decomposition time of 0.74 years and high lignin-to-nitrogen ratios constrain readily decomposable substrates and nutrient mineralization (Giweta, 2020; Xu et al., 2024).

Albizia procera (S4) recorded the lowest MBC values across all periods (pre-monsoon: 43.53 ± 4.70 to 72.02 ± 4.74 µg C g^{-1}; monsoon: 66.50 ± 6.41 to 94.66 ± 5.52 µg C g^{-1}) (Zhao et al., 2020), indicating $p<0.05$. The inferior performance reflects poor litter quality, limited root exudate diversity, and reduced rhizosphere priming effects (Rakotonindrina et al., 2025). The species exhibited pronounced post-monsoon declines (61.25 ± 5.34 to 83.41 ± 6.46 µg C g^{-1}), demonstrating insufficient substrate availability during moisture-stressed conditions (Singh et al., 2025). Shallow-rooted species produce fewer diverse root exudates and maintain lower belowground carbon allocation (Mori et al., 2016).

However, these values still lag behind the Natural Soil value of 157.59 ± 8.26 to 170.56 ± 12.12 µg C/g in all three seasons, indicating that while soil function is improving, full recovery may require more time or additional interventions. Species-wise, S1 consistently showed higher MBC than other species, suggesting species differences in litter quality, root exudation, or canopy cover that influence microbial habitat and carbon availability (Fig. 2).

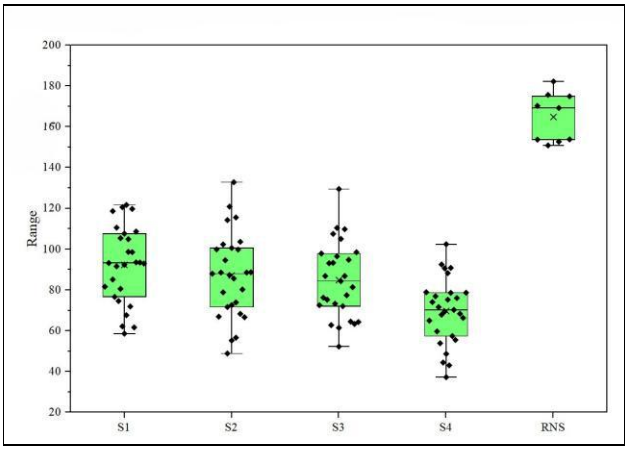

Fig 2. Species-specific variation in soil microbial biomass carbon (MB-C, µg C g^{-1}) across four plantation tree species and comparison with reference normal soil (RNS) at 15–30 cm depth. Code: S1= Dalbergia sissoo, S2= Azadirachta indica, S3= Tectona grandis, S4= Albizia procera. Abbreviation: RNS= Reference Normal Soil.

3.2 Seasonal Dominance and Environmental Controls

MBC values ranged 70–130 µg C g^{-1} during monsoon (median ~95 µg C g^{-1}) versus 40–110 µg C g^{-1} pre-monsoon, reflecting moisture availability as the primary environmental driver creating two-fold increases during optimal conditions (Barbhuiya et al., 2004; Hao et al, 2025) (Fig. 3). Pre-monsoon depression (40–110 µg C g^{-1}) reflects combined stress from reduced soil moisture, elevated temperatures, and substrate limitation (Lyngdoh & Karmakar, 2018). Intermediate post-monsoon values (60–110 µg C g^{-1}) indicate gradual biomass decline during seasonal transition (Sarkar et al., 2024). Greater pre-monsoon variability suggests substantial heterogeneity in soil microhabitats across plantation sites, while condensed monsoon distributions indicate homogenizing effects of abundant moisture (Bolat et al., 2022; Mori et al., 2016). Moisture-induced community shifts from drought-tolerant taxa to metabolically active populations during moist conditions have implications for nutrient cycling efficiency (Singh et al., 2025; Rakotonindrina et al., 2025).

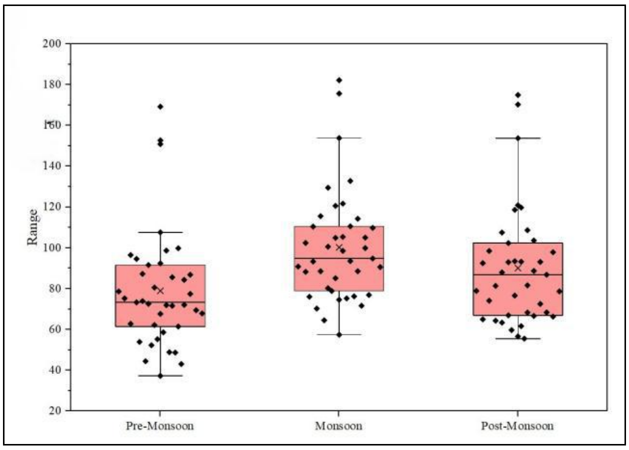

Fig 3. Seasonal variation in soil microbial biomass carbon (MB-C, µg C g^{-1}) at 15–30 cm depth across pre-monsoon, monsoon, and post-monsoon periods at Nandani Limestone Mines.

3.3 Chronosequence Analysis: Plantation Age Gradient and Soil Microbial Biomass Recovery

Five-year-old plantations showed lowest MBC (mean ~68 µg C g^{-1}) with extensive variability indicating species-based heterogeneity during early establishment. Mean MBC approximately doubled from 5-year (68 µg C g^{-1}) to 15-year plantations (85 µg C g^{-1}), demonstrating substantial microbial recruitment during early-to-middle development stages (Fig. 4). Twenty-five-year plantations approached 60% recovery toward reference soil (171 µg C g^{-1}) with mean ~100 µg C g^{-1}, yet remained ~40% depleted relative to natural soils, indicating restoration timelines may exceed standard rotation periods in severely disturbed environments (He et al., 2024; Xu et al., 2018). RNS values (170–180 µg C g^{-1}) represent the recovery target and emphasize mining-induced degradation extent. Tighter value distributions in mature plantations suggest greater microbial community stability and resistance with ecosystem maturation.

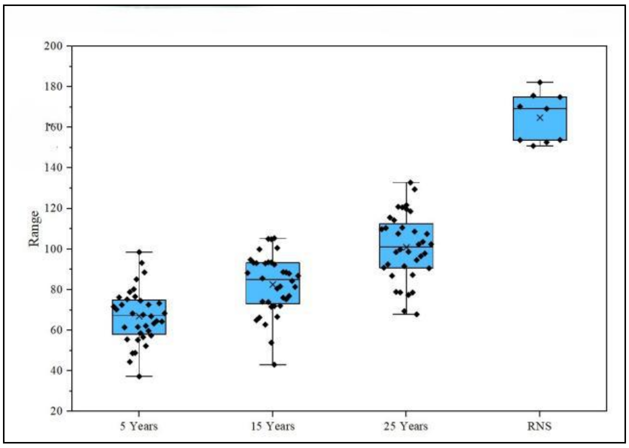

Fig 4. Chronosequence analysis of soil microbial biomass carbon (MB-C, µg C g^{-1}) recovery across plantation age classes and comparison with reference normal soil (RNS) at 15–30 cm depth. Abbreviation: RNS= Reference Normal Soil.

3.4 Multivariate Integration: Three-Way Interactions Among Age, Species, and Seasonality

Quantitative MBC data (Table 1) remained substantially below natural soil levels (157.59–170.56 µg C g^{-1}) across all seasons, indicating incomplete restoration requiring extended timelines or supplementary interventions. The overwhelming influence of seasonal moisture dynamics emerged as the primary environmental driver, with species-wise differences in litter quality and root exudation significantly modulating age-related recovery patterns. Complex interactions between seasonal moisture availability, species-specific litter chemistry, root architecture, and rhizosphere exudate composition collectively determine restoration success (Rakotonindrina et al., 2025).

Three-way analysis of variance (ANOVA) revealed that plantation age, tree species, and season were all significant main effects on soil microbial biomass carbon (F[2,72]=137.87, F[3,72]=33.75, and F[2,72]=57.67, respectively; all p<0.001). Plantation age emerged as the dominant factor, explaining 47.4% of total variance in MB-C concentrations, reflecting the strong recovery trajectory across the chronosequence (Table 2). Season explained 19.8% of variance, demonstrating that seasonal moisture availability substantially constrains microbial biomass dynamics. Species identity explained 17.4% of variance, indicating significant but subordinate effects relative to age and season. Notably, all two-way and three-way interaction terms were non-significant (p>0.36), suggesting that age, species, and seasonal effects operate independently without complex interactive effects. These additive relationships indicate that soil microbial biomass recovery follows predictable, generalizable patterns determined primarily by chronological age progression, with secondary modulation by seasonal environmental constraints and species-specific characteristics. The model explained 84.7% of total variance with only 12.4% residual error, demonstrating robust factorial design and strong predictive capacity for ecosystem recovery trajectories.

Table 2. Three-way ANOVA summary table showing the main and interaction effects of tree species, plantation age, and season on soil microbial biomass carbon at 15–30 cm depth.

	df	***Sum of Squares***	***Mean Square***	***F Value***	***P Value***
Spp	3	7627.831	2542.61	33.74765	<0.001***
Age	2	20775.09	10387.54	137.8721	<0.001***
Season	2	8689.73	4344.865	57.66867	<0.001***
Spp * Age	6	496.7257	82.78762	1.09883	0.37154*ns*
Spp * Season	6	164.4743	27.41239	0.36384	0.89944*ns*
Age * Season	4	117.0085	29.25211	0.38826	0.81638*ns*
Spp * Age * Season	12	501.9442	41.82869	0.55519	0.87027*ns*
Error	72	5424.614	75.34186		
Corrected Total	107	43797.41			

Hierarchical clustering dendrogram analysis revealed pronounced primary separation with reference soil (RNS) clustering distinctly at the upper range (180–183 µg C g^{-1}) in intense red coloration, emphasizing the substantial ecological distinction between natural and reclaimed soils (He et al., 2024; Ogola et al., 2021). Early treatments (5-year plantations) occupied the left region with dark blue coloration (~37–40 µg C g^{-1}), indicating heterogeneous recovery trajectories across species (Fig. 5). Progressive colour gradients from blue (young) through intermediate colours (middle-aged) to red tones (mature) indicate smooth chronological progression in microbial biomass accumulation (He et al., 2024). Monsoon samples clustered more closely regardless of species identity, demonstrating that optimal moisture creates convergent environmental selection pressures overriding species-specific differences (He et al., 2024; Ogola et al., 2021). This pattern indicates that plantation age and seasonality represent dominant organizing principles for microbial composition, while species identity exerts subordinate but significant modulatory effects.

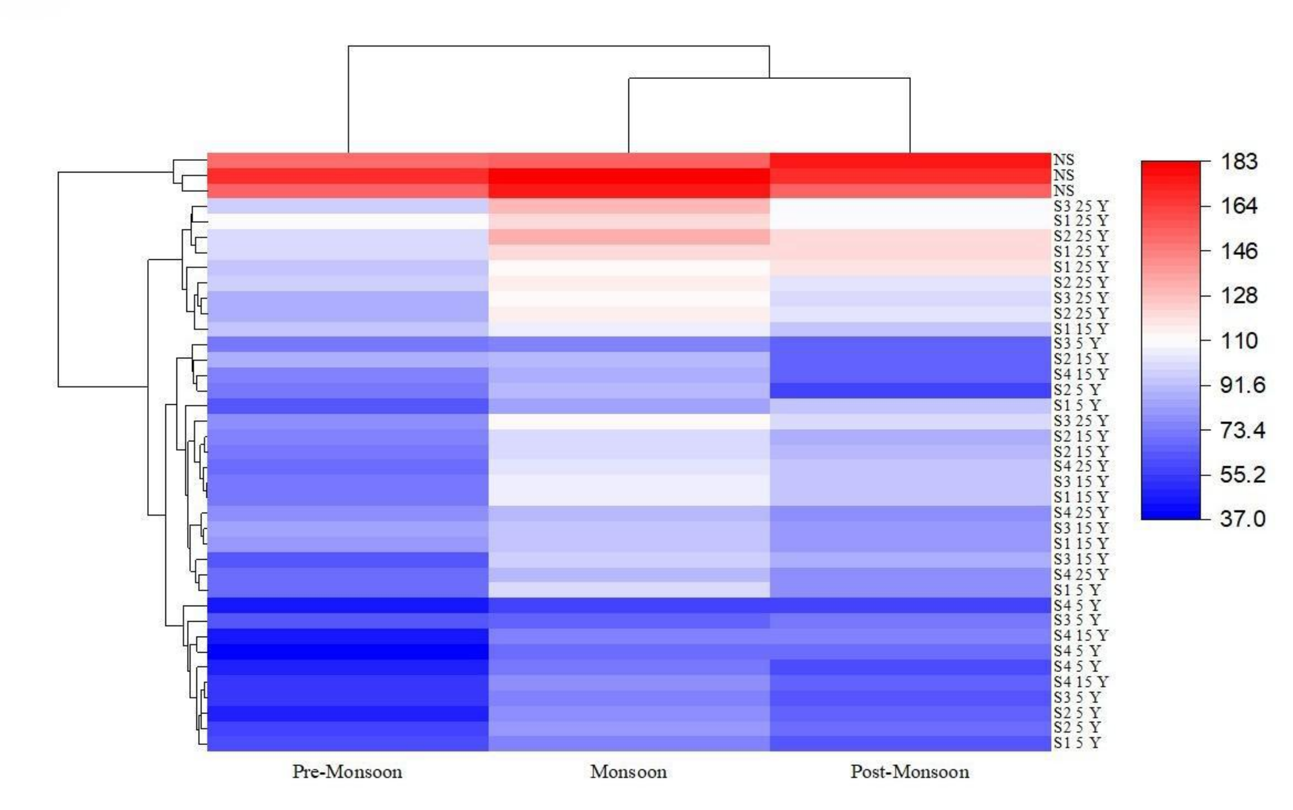

Fig 5. Hierarchical clustering dendrogram with color-coded heatmap of soil microbial biomass carbon (MB-C, µg C g^{-1}) across all treatment combinations at Nandani Limestone Mines. Code: S1= Dalbergia sissoo, S2= Azadirachta indica, S3= Tectona grandis, S4= Albizia procera. Abbreviation: NS= Normal Soil.

The complex interplay of factors reveals that species effects are conditional on seasonal context and plantation age. Legume-dominated plantations (*D. sissoo*, *A. indica*) outperform under both optimal and stress conditions, while timber species show greater seasonal dependency. Advanced molecular techniques reveal moisture-induced shifts from drought-tolerant to metabolically active taxa with implications for nutrient cycling (Singh et al., 2025; Rakotonindrina et al., 2025).

IV. CONCLUSION

This study demonstrates that forest plantations markedly enhance soil microbial biomass carbon in reclaimed limestone mine spoils, with both species identity and stand age exerting significant influence. *Dalbergia sissoo* and *Azadirachta indica* consistently supported higher microbial biomass across all seasons and ages, reflecting their labile litter inputs, diverse root exudates, and deep rooting systems that sustain subsurface carbon supply. *Tectona grandis* showed moderate improvement, constrained by recalcitrant litter chemistry, whereas *Albizia procera's* shallow roots and poorer litter quality yielded the lowest microbial recovery. Seasonal dynamics further modulated these trends, with monsoon moisture driving peak microbial activity and biomass, underscoring the critical role of soil water availability in post-mining restoration. The natural soil control highlighted that, although plantation-driven recovery significantly improves microbial biomass, it remains below undisturbed forest levels. Integrating fast-growing, high-quality litter species like *D. sissoo* and *A. indica*, coupled with moisture-conserving amendments or mixed-species plantings, offers a promising strategy for accelerating microbial and overall ecosystem recovery in degraded limestone mine landscapes.

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