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Estimation of enzymatic activities under various forest tree species as index of mine soil genesis in chronosequence limestone mine spoils

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Abstract
Mining activities significantly disrupt soil properties, necessitating ecological restoration efforts for sustainable land rehabilitation. Soil enzyme activities serve as vital indicators of soil genesis and restoration progress, reflecting microbial functions and nutrient cycling processes. This study assesses enzymatic activities under various forest tree species across a chronosequence of limestone mine spoils in Nandini Limestone Mine, Durg, Chhattisgarh. Four dominant tree species namely *Dalbergia sissoo, Azadirachta indica, Tectona grandis,* and *Albizia procera* were selected for evaluation at three plantation age gradations (5, 15, and 25 years). Soil samples were collected from rhizospheric regions and analyzed for invertase, phosphatase, urease, and dehydrogenase activities to determine their role in mine soil genesis and restoration. Results indicate that enzymatic activities improve with plantation age, signifying progressive soil restoration. Among the species, *Dalbergia sissoo* exhibited the highest enzymatic activity across all parameters, closely followed by *Azadirachta indica*. *Albizia procera* showed moderate enhancement, while *Tectona grandis* demonstrated the lowest enzyme activity, indicating limited contribution to soil rehabilitation. Invertase activity, critical for carbon cycling, increased significantly over time, with *Dalbergia sissoo* exhibiting the highest enhancement (937.39 µg/g soil/hour at 25 years). Phosphatase, a key enzyme in phosphorus mobilization, showed similar trends, reaching a peak of 172.05 µg PNP/g soil/hour under *Dalbergia sissoo*. Urease activity, crucial for nitrogen cycling, was highest in *Dalbergia sissoo* (46.89 µg NH_4-N/g soil/hour at 25 years), followed by *Azadirachta indica*. Dehydrogenase activity, an indicator of microbial metabolic activity, was highest under *Azadirachta indica*, suggesting its significant role in promoting microbial proliferation. The correlation data suggests that enzymatic activities in younger soils are highly interconnected ($p<0.01$), reflecting rapid microbial and organic matter turnover and as plantations matures, the interdependence of enzyme functions slightly declines. These findings highlight the importance of tree species selection in mine spoil restoration. *Dalbergia sissoo* emerges as the most effective species for enhancing soil biochemical properties, making it a strong candidate for ecological restoration. The study underscores the role of soil enzyme activity as a robust bioindicator of soil quality and restoration success, providing valuable insights for afforestation and land reclamation initiatives in degraded mining landscapes.

Keywords: Soil enzymes, restoration, mine soil, phytoremediation, chronosequence, tree species

Introduction
Soil serves as a fundamental medium composed of an unconsolidated matrix of minerals, organic matter, microorganisms, and nutrients, all of which collectively influence its physico-chemical and biological properties. These characteristics play a critical role in shaping soil microbial populations, enzymatic activities, and overall soil health. The capacity of soil to support plant growth is largely determined by processes such as mineralization, microbial proliferation, and organic matter decomposition, which in turn enhance nutrient availability and soil fertility [1, 2]. As a substrate for plant development, soil exhibits a complex matrix of organic and inorganic components, with distinct chemical, physical, and biological characteristics that vary spatially and temporally, extending from the Earth's surface to its deeper layers. The formation of soil is governed by pedogenic processes, wherein parent rock material undergoes weathering through mechanical, chemical, and biological interactions over extended periods [3, 4]. Mining activities, which involve extensive excavation and removal of topsoil, significantly disrupt the natural landscape and degrade

soil structure. Such anthropogenic disturbances lead to alterations in microbial communities, loss of organic matter, reduction in soil fertility, and changes in ecosystem functionality [5, 6, 7]. Soil contamination due to heavy metals and toxic residues further exacerbates environmental degradation, impacting soil microbial biomass and enzymatic activities, which are vital indicators of soil recovery and ecological restoration [8].

The assessment of soil enzymatic activities is crucial for monitoring and maintaining soil quality, as these biochemical markers play an essential role in nutrient cycling and organic matter decomposition in terrestrial ecosystems [9, 10]. Soil enzymes, such as dehydrogenases, phosphatases, ureases, and β-glucosidases, contribute to key biochemical reactions that facilitate organic matter decomposition, nitrogen mineralization, and phosphorus mobilization [11, 12]. These enzymatic activities, influenced by microbial dynamics and soil physicochemical properties, serve as reliable biomarkers for evaluating soil health and ecosystem resilience, particularly in mine-affected landscapes [13, 14]. Moreover, soil structural stability is maintained through the synergistic actions of microbial activity and enzymatic contributions, which enhance aggregation, promote soil porosity, and regulate water retention [15]. Understanding soil enzymatic functions can provide valuable insights into soil restoration strategies, facilitating the rehabilitation of degraded lands through biological and ecological interventions, such as bioremediation and phytoremediation [16, 17].

Additionally, soil enzyme activities can reveal minute alterations in soil quality before the latter are detected by other means of soil analysis [18]. In the context of mine spoil restoration, periodic monitoring of soil enzyme activities is essential to evaluate the progress of tree-based reclamation strategies and their impact on soil genesis over time [19]. Tree planting on mine spoils not only stabilizes the soil structure but also enhances microbial activity and organic matter accumulation, which are critical for the development of a functional soil ecosystem [4, 20]. Over time, these biological processes contribute to the formation of a stable soil matrix, improved nutrient availability, and increased biodiversity, all of which are essential for sustainable ecosystem recovery [21]. Evaluating the success of eco-restoration efforts in degraded mining landscapes is crucial for understanding the effectiveness of restoration strategies and identifying areas for improvement [22]. Long-term monitoring of soil enzyme activities, along with other physicochemical and biological parameters, provides a comprehensive assessment of soil restoration progress and ecosystem functionality [23]. Such evaluations help refine restoration techniques, ensuring that they are tailored to the specific ecological conditions of the site and promoting the long-term sustainability of the restored ecosystems [24].

Moreover, integrating soil enzyme activity data with other ecological indicators, such as plant diversity, microbial biomass, and soil organic carbon, can provide a holistic understanding of the restoration trajectory [25, 26]. This multidisciplinary approach not only enhances the accuracy of restoration assessments but also contributes to the development of best practices for mine soil rehabilitation, ultimately supporting global efforts to restore degraded lands and mitigate environmental impacts [27, 28]. The present study deals with the multivariate profiling of different age series tree species growing on degraded limestone mine soil based on enzymatic characterization with site descriptions to interpret data in explaining the differential pattern of changes in mine soil in reference to forest tree species, which provide valuable inputs for the assessment of mine soil genesis and impact of various tree vegetation on them.

Materials and Methods

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 (Figure 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.

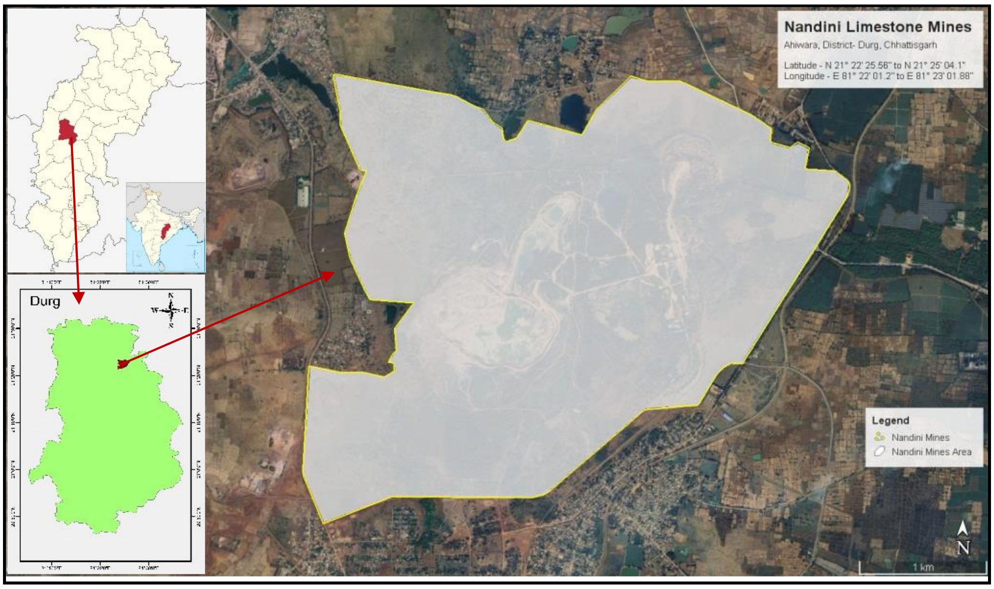

Fig 1: Geographical location of Nandini Limestone Mines (970 hectares) in Durg, Chhattisgarh.

Selection of Sampling Plots

For the purpose of this study, soil samples were collected from the rhizospheric region of four dominating forest tree species (based on IVI) at three age gradations in the Nandini Limestone Mine. The entire area was first divided into twelve segments based on the availability of pure plantations of selected forest tree species namely S1-*Dalbergia sisoo*, S2-*Azadirachta indica*, S3-*Tectona grandis* and S4-*Albizia procera* at three age gradations (A-5 years, B-15 years and C-25 years). Then soil samples from each plantation segment were randomly collected at 0-15 cm soil depth and a total of 36 composite soil samples (with replications) were collected from these plots. Soil samples from one plot was collected in the nearby natural forest site which was taken as control to understand the restoration progress in chronosequence mine spoils.

Soil Sampling Method

Sampling was done in accordance with general microbiological protocol from 12 different points around the marked opencast limestone mining area at three age gradations in monsoon season as enzymatic activities are found superior in monsoon when compared to other seasons. Each sampling point was divided into 3 blocks, and from each block soil samples were collected randomly at 0-15 cm soil depth by digging pits of (15 x 15 x 15) cm^3 size. Samples collected from each block were referred as 'sub-samples', and was thoroughly mixed to form one 'composite sample'. Unwanted debris, forest litter, stones, and gravels were removed from the samples and then the composite sample was replicated thrice for further analysis and data collection. Similar sampling strategies was followed for all the 12 sampling points and the nearby natural forest soil. After collection, the samples were subjected to sieving (0.2 mm mesh size) and stored at 4 °C until analysed.

Methodology for Analysis of Enzymes

Invertase activity of different limestone mine overburden spoil as well as nearby forest soil samples were determined by spectrophotometric method by taking absorbance at 540 nm [29] by using sucrose as substrate and incubated at 37 °C for 24hr. Phosphatase activity was determined by calorimetric estimation of p-nitrophenol released when soil is incubated with buffered sodium p-nitrophenyl phosphate solution and toluene at 37 °C for 1hr [30]. Urease activity of different age series iron mine overburden spoil as well as NF soil were determined by titration method using 0.005N NH_2S0_4 with boric acid indicator [31] using urea as substrate. Dehydrogenase activity was estimated through the reduction of 2, 3, 5-triphenylotetrazolium chloride (TTC) as an electron acceptor to red-coloured triphenyl formazon (TPF), which was determined by spectrophotometric method by taking absorbance at 485 nm [32].

Statistical Analysis

In the present study, Statistical analyses (Mean and Standard Deviation) have been calculated using Microsoft Excel 2021. The simple correlation analysis, Duncan's multiple range test and hierarchical clustering between different soil properties was performed using SPSS V25 software.

Method of Correlation

The Karl Pearson correlation method was used to measure the strength of the relationship between the two numeric and continuous variables. Since the results of the parameters were continuous and normally distributed, therefore, the Pearson correlation test was used for the analysis of soil parameters.

Method of Hierarchical Clustering

A Hierarchical Cluster Analysis was conducted to explore the relationship between four tree species S1 (*Dalbergia sissoo*), S2 (*Azadirachta indica*), S3 (*Tectona grandis*), and S4 (*Albizia procera*) and natural forest soil (NS) based on their similarity. This analysis provides valuable insights into the soil characteristics and ecological connections among these species [33], helping to understand the process of mine soil genesis over time in chronosequence spoils.

Results and Discussion

Table 1 provides a comprehensive comparison of various enzymatic activities of mine spoil under different plantations (S1-*Dalbergia sisoo*, S2-*Azadirachta indica*, S3-*Tectona grandis* and S4-*Albizia procera*) at different age gradations (A-5 years, B-15 years and C-25 years) were studied in which invertase enzyme ranged from 81.40 to 937.39 µg/g soil/hour, while the phosphatase enzyme ranged from 67.96 to 172.05 µg PNP/g soil/hour in plantation species. Also, the urease and dehydrogenase enzymes were studied resulting in ranges from 8.15 to 46.89 µg NH4-N/g soil/hour and 0.741 to 4.420 µg TPF/g soil/hour respectively in plantation species. Whereas in natural forest soil, invertase enzyme reached 1064.92 µg/g soil/hour, and the phosphatase enzyme reached 188.10 µg PNP/g soil/hour. Also, the urease and dehydrogenase enzymes reached 57.63 µg NH4-N/g soil/hour and 4.868 µg TPF/g soil/hour respectively in natural forest soil.

Invertase Activity

Invertase activity, critical for carbon cycling by breaking down sucrose into glucose and fructose, shows a marked increase with plantation age across all species [34, 20]. *Dalbergia sissoo* exhibited the most significant impact on invertase activity, starting at 107.74 µg/g soil/hour in 5-year-old plantations and peaking at 937.39 µg/g soil/hour in 25-year-old plantations (Figure 2). This steady and pronounced increase underscores the species' ability to enhance soil organic matter decomposition, likely due to its high-quality litter and root exudates that stimulate microbial activity [4]. The dominance of *Dalbergia sissoo* in promoting carbon metabolism aligns with studies that highlight its effectiveness in improving soil fertility and microbial diversity in degraded landscapes [21]. *Azadirachta indica* followed a similar trend but with comparatively lower values, reaching 883.32 µg/g soil/hour at 25 years. While *Azadirachta indica* is known for its soil health-promoting properties, its moderate invertase activity suggests a less pronounced impact on carbon cycling compared to *Dalbergia sissoo*. This could be attributed to differences in litter decomposition rates or microbial community composition in its rhizosphere [35]. Nevertheless, its consistent performance highlights its potential as a valuable species for mine soil restoration.

Tectona grandis consistently demonstrated the lowest invertase activity, with a maximum of 809.24 µg/g soil/hour at 25 years. This limited contribution to carbon cycling may be due to its recalcitrant leaf litter, which decomposes slowly and provides less substrate for microbial activity [4].

Additionally, the microbial interactions in its rhizosphere may differ from those of other species, further limiting its impact on soil enzyme activities [36]. These findings suggest that *Tectona grandis* may be less effective in enhancing soil biological functions compared to other species. *Albizia procera* performed better than *Tectona grandis* but remained behind *Dalbergia sissoo* and *Azadirachta indica*, with invertase activity reaching 866.95 µg/g soil/hour at 25 years (Figure 2). This moderate performance reflects its potential to contribute to soil organic matter decomposition, likely influenced by its intermediate litter quality and microbial associations [19]. While not as effective as *Dalbergia sissoo*, *Albizia procera* still offers significant benefits for soil restoration, particularly in mixed-species plantations.

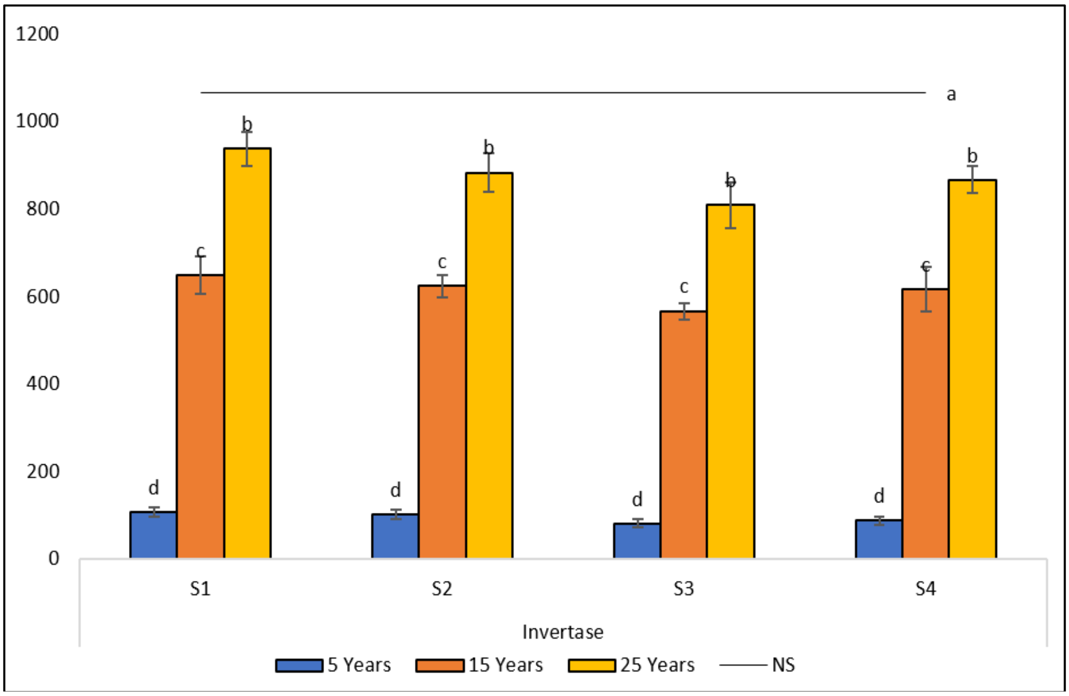

Fig 2: Activity of invertase enzyme under four different plantation species; *Dalbergia sissoo* (S1), Azadirachta indica (S2), *Tectona grandis* (S3), and *Albizia procera* (S4) as well as natural forest soil (NS) in different age gradations.

Phosphatase Activity

Phosphatase activity, a crucial indicator of phosphorus availability through organic phosphorus mineralization, also increases with plantation age, albeit with variations among species [37, 38]. *Dalbergia sissoo* demonstrates the highest phosphatase activity levels, starting at 89.32 µg PNP/g soil/hour in 5-year-old plantations and rising to 172.05 µg PNP/g soil/hour in 25-year-old plantations. This trend highlights the species' exceptional ability to enhance phosphorus mobilization, which is critical for improving soil fertility and supporting plant and microbial communities in degraded ecosystems [20, 21]. The near-natural forest levels of phosphatase activity (188.10 µg PNP/g soil/hour) achieved by *Dalbergia sissoo* underscore its potential as a key species for restoring phosphorus cycling in mine-degraded soils [39]. *Azadirachta indica* shows a similar increasing trend in phosphatase activity, peaking at 150.20 µg PNP/g soil/hour at 25 years (Figure 3). While this reflects moderate efficacy in improving phosphorus cycling, it is slightly lower than that of *Dalbergia sissoo*. This difference may be attributed to variations in litter quality, root exudates, or microbial community composition, which influence organic phosphorus mineralization [16, 40]. Despite this, *Azadirachta indica* remains a valuable species for enhancing soil phosphorus availability in restoration projects, particularly in mixed-species plantations.

Albizia procera exhibits consistently lower phosphatase activity, peaking at 114.07 µg PNP/g soil/hour at 25 years, though it shows higher activity (75.82 µg PNP/g soil/hour) than *Tectona grandis* at the 5-year mark. This limited activity may result from slower nutrient turnover rates and potentially lower microbial activity in the soil under *Albizia procera* plantations [19, 38]. While it contributes to phosphorus dynamics, its impact is less pronounced compared to *Dalbergia sissoo* and *Azadirachta indica*. *Tectona grandis*, though not as effective as *Dalbergia sissoo* or *Azadirachta indica*, performs better than *Albizia procera*, reaching a maximum phosphatase activity of 117.70 µg PNP/g soil/hour (Figure 3). This moderate performance suggests that *Tectona grandis* can contribute to phosphorus cycling, albeit less robustly than other species. Its recalcitrant leaf litter and slower decomposition rates may limit its ability to enhance phosphorus availability [4, 39]. However, its inclusion in mixed-species plantations could still provide benefits for soil restoration, particularly in combination with species that exhibit higher phosphatase activity.

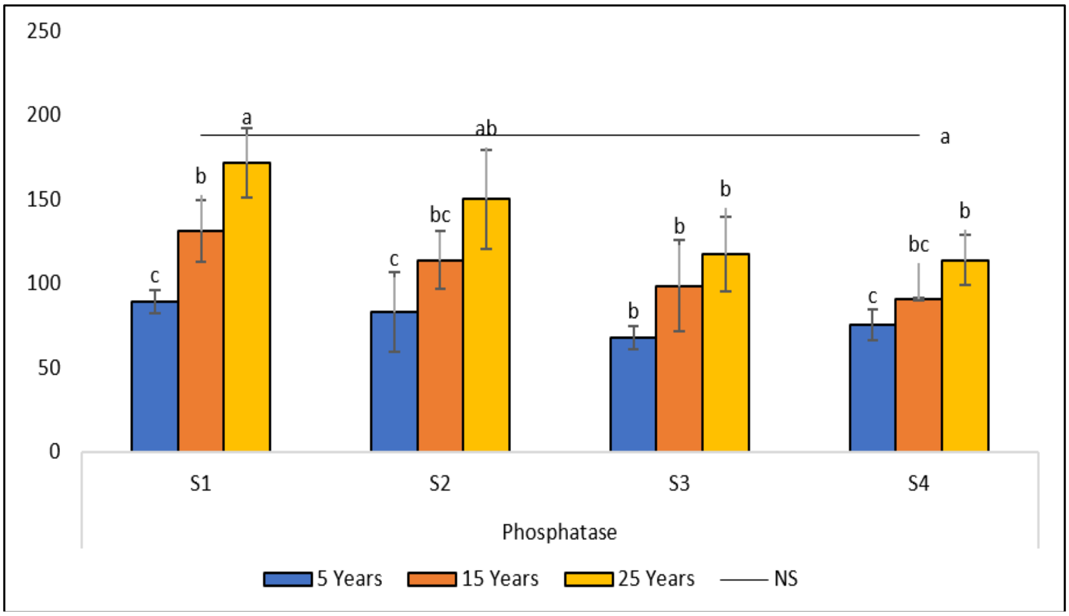

Fig 3: Activity of phosphatase enzyme under four different plantation species; *Dalbergia sissoo* (S1), Azadirachta indica (S2), *Tectona grandis* (S3), and *Albizia procera* (S4) as well as natural forest soil (NS) in different age gradations.

Urease Activity

Urease activity, which facilitates nitrogen cycling by hydrolyzing urea into ammonia and carbon dioxide, is another critical indicator of soil health [38, 40]. *Dalbergia sissoo* demonstrates the highest urease activity, starting at 10.41 µg NH4-N/g soil/hour in 5-year-old plantations and reaching 46.89 µg NH4-N/g soil/hour in 25-year-old plantations (Figure 4). This significant increase underscores the species' strong potential to enhance nitrogen transformation, which is critical for improving soil fertility and supporting plant and microbial communities [21, 39]. The near-natural forest levels of urease activity (57.63 µg NH4-N/g soil/hour) achieved by *Dalbergia sissoo* further emphasize its effectiveness in restoring nitrogen cycling in degraded soils. *Azadirachta indica* and *Albizia procera* show similar trajectories in urease activity but with slightly lower maxima of 42.75 µg NH4-N/g soil/hour and 37.21 µg NH4-N/g soil/hour, respectively, at 25 years. This indicates moderate improvement in soil nitrogen cycling, likely due to their ability to support microbial activity and organic matter accumulation [35, 20]. While these species contribute to nitrogen dynamics, their impact is less pronounced compared to *Dalbergia sissoo*, possibly due to differences in litter quality or microbial community composition [40]. *Tectona grandis* exhibits the lowest urease activity among the species, peaking at 32.93 µg NH4-N/g soil/hour at 25 years, though it shows higher activity (17.55 µg NH4-N/g soil/hour) than *Albizia procera* at the 15-year mark (Figure 4). This limited performance suggests that *Tectona grandis* is less effective in enhancing nitrogen transformation, possibly due to lower microbial biomass or activity in its soil [4, 39]. Its recalcitrant leaf litter and slower decomposition rates may further limit nitrogen availability, making it a less suitable species for improving nitrogen cycling in degraded soils.

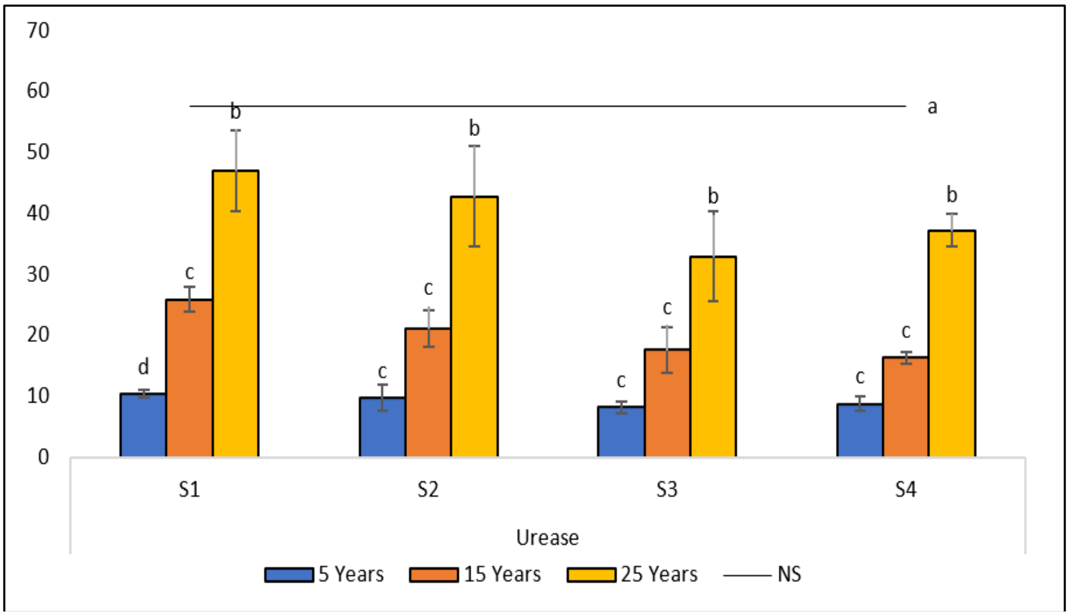

Fig 4: Activity of Urease enzyme under four different plantation species; *Dalbergia sissoo* (S1), Azadirachta indica (S2), *Tectona grandis* (S3), and *Albizia procera* (S4) as well as natural forest soil (NS) in different age gradations.

Dehydrogenase Activity

Dehydrogenase activity, a direct measure of overall microbial activity and soil health, reveals similar increasing trends with plantation age. *Azadirachta indica* (S1 species) after natural soil (4.868 µg TPF/g soil/hour) exhibits the most significant enhancement, starting at 0.915 µg TPF/g soil/hour at 5 years and reaching 4.420 µg TPF/g soil/hour at 25 years (Figure 5). This remarkable increase underscores S2 species' ability to stimulate microbial proliferation, essential for nutrient cycling and organic matter decomposition. The high dehydrogenase activity in *Azadirachta indica* may be attributed to its ability to release root exudates that promote microbial growth and enhance soil enzyme activities, as observed in recent studies [16]. *Dalbergia sissoo* (S2 species) closely follows S1 species, particularly in early stages (0.876 µg TPF/g soil/hour), with activity reaching 4.411 µg TPF/g soil/hour at 25 years, reflecting its strong yet slightly lesser microbial support compared to S1 species. The robust performance of *Dalbergia sissoo* can be linked to its nitrogen-fixing ability, which enriches soil fertility and supports microbial communities [25]. However, its slightly lower dehydrogenase activity compared to *Azadirachta indica* suggests differences in the quality and quantity of organic matter input or root exudation patterns.

Tectona grandis (S3 species) has the lowest dehydrogenase activity, peaking at 3.723 µg TPF/g soil/hour at 25 years (Figure 5). This subdued microbial stimulation may be attributed to slower litter decomposition or lower rhizospheric activity, as *Tectona grandis* is known to produce recalcitrant litter that decomposes slowly, limiting microbial activity [41]. Additionally, its allelopathic properties might inhibit microbial proliferation, as reported in recent studies [8]. *Albizia procera* (S4 species) outperforms S3 species but remains behind S1 and S2 species, with activity increasing to 4.147 µg TPF/g soil/hour. The moderate dehydrogenase activity in *Albizia procera* may be due to its ability to improve soil organic carbon and nutrient availability, though it may not match the microbial stimulation capacity of *Azadirachta indica* or *Dalbergia sissoo* [42].

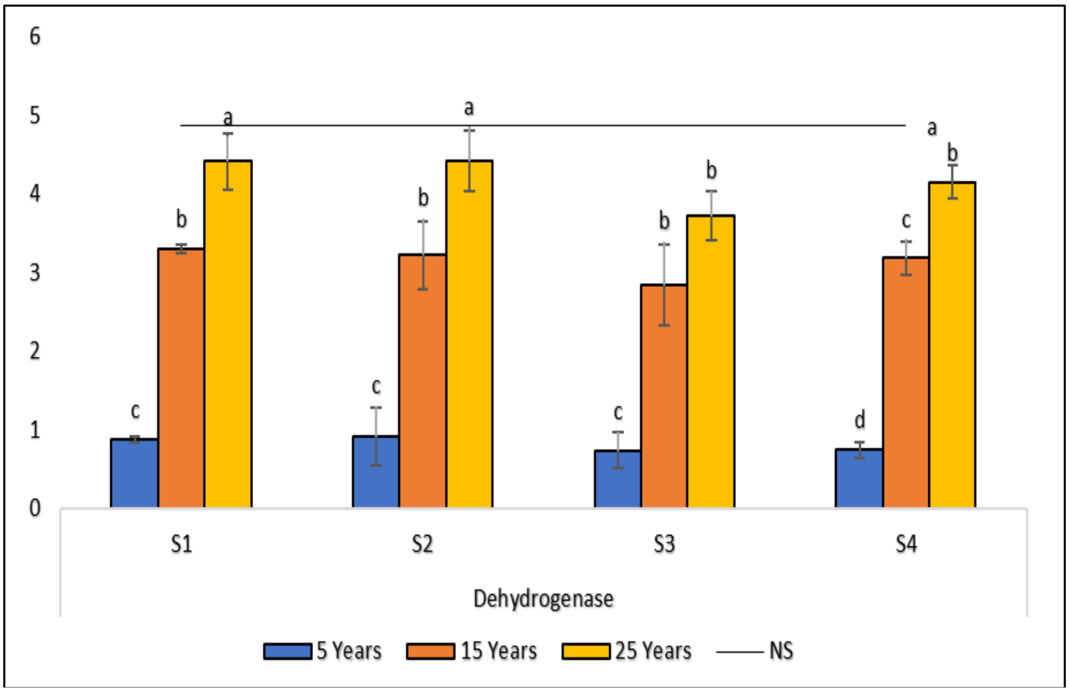

Fig 5: Activity of dehydrogenase enzyme under four different plantation species; *Dalbergia sissoo* (S1), Azadirachta indica (S2), *Tectona grandis* (S3), and *Albizia procera* (S4) as well as natural forest soil (NS) in different age gradations.

Table 1: Various enzymatic properties for soil under four plantation species S1, S2, S3 and S4 as well as natural forest soil (NS) in different age gradations.

Enzyme	Species	Age Gradation			NS	F Value
		5 Years	15 Years	25 Years		
Invertase (μg/g soil/hour)	S1	107.74±10.61 ^{d}	648.16±42.85 ^{c}	937.39±39.31 ^{b}	1064.92±45.54 ^{a}	248.51***
	S2	102.08±11.38 ^{d}	623.83±24.80 ^{c}	883.32±43.85 ^{b}		250.37***
	S3	81.40±9.59 ^{d}	567.24±18.85 ^{c}	809.24±52.23 ^{b}		227.76***
	S4	88.05±9.43 ^{d}	617.33±50.51 ^{c}	866.95±30.32 ^{b}		194.04***
Phosphatase (μg PNP/g soil/hour)	S1	89.32±6.59 ^{c}	131.50±18.56 ^{b}	172.05±20.43 ^{a}	188.10±22.50 ^{a}	14.92**
	S2	83.00±23.59 ^{c}	114.05±17.26 ^{bc}	150.20±29.27 ^{ab}		11.3**
	S3	67.96±6.78 ^{b}	98.79±27.09 ^{b}	117.70±22.31 ^{b}		12.77**
	S4	75.82±9.27 ^{c}	90.94±1.09 ^{bc}	114.07±14.90 ^{b}		25.2***
Urease (μg NH_4-N/g soil/hour)	S1	10.41±0.67 ^{d}	25.89±1.99 ^{c}	46.89±6.61 ^{b}	57.63±4.77 ^{a}	56.97***
	S2	9.75±2.07 ^{c}	21.11±2.92 ^{c}	42.75±8.22 ^{b}		38.76***
	S3	8.15±1.05 ^{c}	17.55±3.77 ^{c}	32.93±7.35 ^{b}		39.08***
	S4	8.77±1.12 ^{c}	16.29±0.93 ^{c}	37.21±2.70 ^{b}		91.94***
Dehydrogenase (μg TPF/g soil/hour)	S1	0.876±0.036 ^{c}	3.306±0.055 ^{b}	4.411±0.364 ^{a}	4.868±0.328 ^{a}	176.34***
	S2	0.915±0.372 ^{c}	3.221±0.437 ^{b}	4.420±0.388 ^{a}		41.23***
	S3	0.741±0.234 ^{c}	2.842±0.513 ^{b}	3.723±0.312 ^{b}		41.63***
	S4	0.745±0.096 ^{d}	3.187±0.210 ^{c}	4.147±0.211 ^{b}		97.72***

Abbreviations: S1-*Dalbergia sissoo*, S2-*Azadirachta indica*, S3-*Tectona grandis*, S4-*Albizia procera*. Values expressed in mean±SD; n = 3. Different letters in a row indicate the significant difference among different age gradation at $p<0.05$ according to Duncan's multiple range tests. (*** Significant at 0.001 level; ** Significant at 0.01 level)

Correlation Analysis

The correlation analysis of enzymatic activities across different aged plantations (5, 15, and 25 years) provides insights into the interdependence of soil enzyme functions over time. The results indicate strong positive correlations among invertase, phosphatase, urease, and dehydrogenase in all plantation ages, with varying levels of statistical significance.

At the youngest plantation (5 years), the correlation values are exceptionally high, exceeding 0.99 for all enzyme pairs (Table 2). Notably, the correlation between invertase and urease (r = 0.99987) and between invertase and dehydrogenase (r = 0.99974) indicates an almost perfect relationship. The phosphatase enzyme also shows a significant correlation with the other enzymes (r > 0.99). These correlations, significant at the $p<0.01$ level, suggest a highly synchronized enzymatic activity in younger soils, possibly due to the rapid decomposition of organic matter and active microbial processes. In the mid-aged plantation (15 years), enzyme correlations remain strong but show a slight decline compared to the 5-year-old soil. While urease and dehydrogenase still maintain a significant correlation above 0.98, the phosphatase correlations decrease slightly (e.g., r = 0.94579 for phosphatase-invertase and r = 0.94485 for phosphatase-dehydrogenase), with some correlations being significant at the $p<0.05$ level. This reduction might indicate a shift in microbial community dynamics and organic matter stabilization over time, affecting enzymatic interactions. In the oldest plantation (25 years), correlations among enzymatic activities show a further decrease (Table 2). While urease and dehydrogenase maintain strong correlations (r = 0.96128, significant at $p<0.01$), the relationships involving phosphatase weaken, with values dropping below 0.90 (e.g., r = 0.88247 for phosphatase-dehydrogenase). The decline in correlation strength suggests a gradual stabilization of soil biochemical processes, where enzyme activities become less interdependent due to changes in nutrient cycling and microbial diversity in mature soils.

Table 2: Simple correlation coefficients (r) between different enzymatic properties for soil at 5, 15 and 25-year-old plantations.

Age	Enzymes	Invertase	Phosphatase	Urease	Dehydrogenase
5 Years	Invertase	1			
	Phosphatase	0.99047**	1		
	Urease	0.99987**	0.99254**	1	
	Dehydrogenase	0.99974**	0.99177**	0.99978**	1
15 Years	Invertase	1			
	Phosphatase	0.94579*	1		
	Urease	0.98839**	0.98107**	1	
	Dehydrogenase	0.99643**	0.94485*	0.98149**	1
25 Years	Invertase	1			
	Phosphatase	0.89645*	1		
	Urease	0.98678**	0.94917*	1	
	Dehydrogenase	0.93824*	0.88247*	0.96128**	1

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level (2-tailed).

Hierarchical Cluster Analysis

The dendrogram illustrates the hierarchical clustering of four tree species and natural forest soil (NS) based on their similarity (Figure 6). S1 (*Dalbergia sissoo*) and S2 (*Azadirachta indica*) cluster first at a rescaled distance of 1.5, indicating strong similarity, likely due to shared soil or ecological requirements. This cluster merges with S3 (*Tectona grandis*) at a distance of 4.5, suggesting moderate similarity. S4 (*Albizia procera*) joins this group at a distance of 4.167, reflecting slightly less similarity compared to the earlier clusters. NS (natural forest soil) forms the most distinct cluster, merging with the tree species group at a distance of 3.2. This indicates that while NS shares some characteristics with the species' associated soils, it represents a broader, more diverse baseline typical of natural ecosystems.

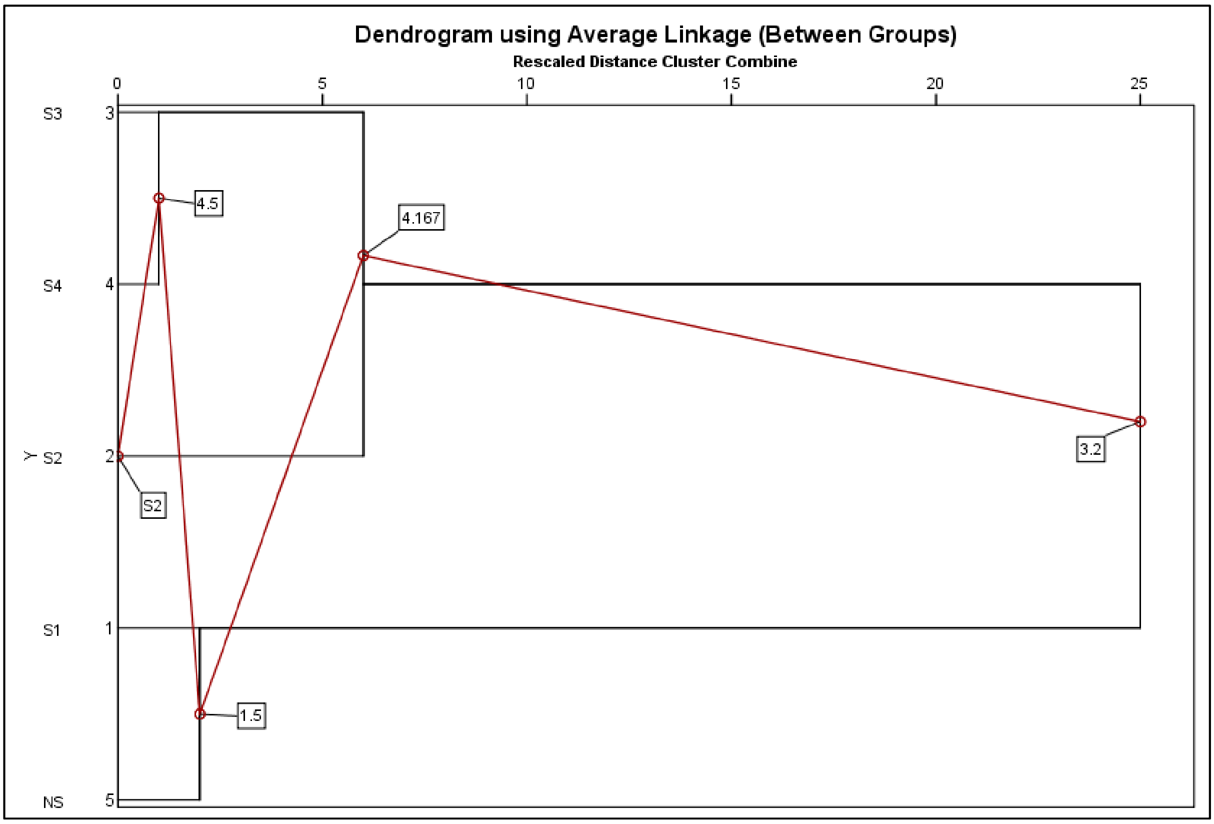

Fig 6: Dendrogram showing average linkage using hierarchical cluster analysis between four different plantation species; *Dalbergia sissoo* (S1), Azadirachta indica (S2), *Tectona grandis* (S3), and *Albizia procera* (S4) as well as natural forest soil (NS) in different age gradations.

Conclusion

The data underscore *Dalbergia sissoo* (S1) as the most effective species across all enzyme activities, enhancing soil health significantly over time. *Azadirachta indica* (S2) emerges as the second most impactful, while *Albizia procera* (S4) offers moderate benefits. *Tectona grandis* (S3) consistently shows the least enhancement, suggesting limited ecological benefits in terms of soil enzyme dynamics. The trends emphasize the importance of species selection and plantation age in promoting soil fertility and microbial health, with S1 standing out as the optimal choice for improving degraded soils. The observed decline in enzymatic interdependence with plantation age can be attributed to several factors. In younger soils, microbial activity is high due to fresh organic matter inputs, leading to strong correlations among enzymes involved in decomposition and nutrient cycling. As the plantation matures, soil organic matter stabilizes, microbial communities diversify, and nutrient availability becomes more regulated, reducing the reliance of one enzyme system on another. Additionally, older plantations may develop a more structured soil profile with increased humification, altering enzyme diffusion and substrate availability. These changes lead to a gradual decoupling of enzymatic interactions, reflecting a shift from rapid nutrient turnover in early stages to a more stable and self-regulated soil ecosystem in mature plantations. The hierarchical cluster analysis helps understand ecological relationships and can guide species selection for afforestation, agroforestry, or restoration projects. It highlights compatibility among species and their potential adaptability to specific soil conditions. These findings suggest that soil enzymatic interactions evolve with age, reflecting changes in microbial communities and nutrient dynamics. Early-stage soils exhibit competitive interactions, while older soils show more cooperative enzymatic relationships, contributing to ecosystem stability and nutrient balance.

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