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Rhizosphere carbon sequestration dynamics in restored mangrove ecosystems

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Abstract
Mangrove ecosystems represent globally significant carbon sinks, with particular importance in their belowground rhizosphere processes, lower DOC alone cannot explain the 41% carbon deficit if productivity is equivalent.
The role of microbial biomass emerges as central to understanding carbon recovery constraints. Microbial biomass carbon was 32% lower in restored forests, and microbial biomass explained more variance in soil carbon stocks than any other single variable (r = 0.68). Structurally, this low MBC could reflect two mechanisms: [1] reduced microbial abundance, limiting total carbon stabilization through microbial necromass; or (2) reduced microbial efficiency in converting labile carbon to stable biomass (20). The microbial quotient analysis provides insight here lower MBC: SOC ratios in restored forests (0.38% vs. 0.51%) suggest that the carbon present is less associated with active microbial biomass, indicating reduced microbial presence relative to carbon stocks. This pattern implies that restored forest rhizospheres support smaller microbial populations, potentially explaining slower carbon accumulation.
The shift toward fungal-dominated communities in restored mangroves, evidenced by increased fungal PLFA proportions and lower bacterial-to-fungal ratios, has implications for carbon cycling. Fungi typically mineralize organic matter more slowly than bacteria and produce more persistent extracellular polymers (21). While this might intuitively favor carbon accumulation, our observation that total microbial biomass (fungal + bacterial) is lower in restored forests suggests that the community shift reflects overall biomass reduction rather than a shift toward more conservative carbon metabolism. The lower B:F ratio may indicate substrate quality changes with restored systems dominated by more recalcitrant substrates preferentially utilized by fungi or reduced nutrient cycling efficiency.
Lower soil respiration rates in restored forests (Figure 2A) indicate reduced heterotrophic microbial activity, consistent with the lower microbial biomass. However, respiration rates increase with restoration age, approaching natural forest levels in the oldest restored sites. This suggests that microbial community establishment and activity gradually recover over time, and that time alone may be sufficient for recovery if ecological constraints are not severe.

Keywords: Mangrove ecosystems, microbial biomass carbon, soil carbon stocks, restored forests, microbial community structure

Introduction

Mangrove ecosystems are among the most carbon-dense terrestrial ecosystems globally, storing between 400-700 Mg C ha^{-1} in soils [1], exceeding carbon stocks in most tropical forests. Despite occupying only 0.1% of global forest area, mangroves contribute disproportionately to coastal carbon sequestration, accumulating soil carbon at rates 5-10 times faster than terrestrial forests [2, 3]. The mechanisms underlying this exceptional carbon sequestration capacity involve multiple interconnected processes: rapid aboveground productivity supplying carbon through litterfall, anaerobic conditions limiting carbon mineralization, and hydrodynamic sorting of refractory organic matter creating highly stable carbon pools [4]. The rhizosphere the soil zone directly influenced by root activity and microbial processes represents the primary locus where carbon sequestration occurs, through both direct carbon allocation via root exudates and indirect processes of organic matter stabilization [5].

Mangrove forest coverage has declined globally by approximately 35% over the past two decades, primarily through conversion to aquaculture, agriculture, and coastal development [6]. These losses are ecologically catastrophic, representing permanent loss of some of Earth's most productive and biodiverse ecosystems. Concurrently, mangrove deforestation represents a significant source of atmospheric carbon, with estimates suggesting that mangrove losses contribute 0.02-0.12 Gt CO_2 $year^{-1}$ to global emissions through both carbon oxidation and foregone sequestration [7]. Consequently, mangrove restoration has emerged as a priority climate mitigation strategy, with thousands of hectares restored annually across Southeast Asia, particularly in Bangladesh, Indonesia, and Vietnam [8].

However, restoration success remains variable and poorly understood, particularly regarding the temporal dynamics of carbon recovery. Most restoration efforts have focused on aboveground productivity and structural recovery (canopy closure, species diversity), while belowground carbon sequestration capacity has received minimal attention [9]. Existing studies comparing restored and natural mangroves report conflicting findings: some demonstrate rapid recovery of soil carbon within 10-15 years post-restoration [10], while others document persistent deficits requiring >30 years for recovery [11]. These divergent findings likely reflect differences in restoration methodology, site-specific edaphic conditions, and measurement approaches. Critically, most studies have quantified bulk soil carbon stocks without examining the rhizosphere processes driving carbon accumulation specifically, the interactions between root production, microbial biomass, and organic matter

turnover that ultimately determine ecosystem carbon sequestration potential [12].

The rhizosphere represents a dynamic interface where plant roots, soil microorganisms, and organic matter interact to shape carbon cycling. Root exudates supply labile carbon directly to microbial communities, while fine root senescence contributes more recalcitrant carbon pools. Rhizosphere microorganisms simultaneously mineralize organic matter and synthesize microbial biomass, creating a balance between carbon loss (respiration) and stabilization (microbial necromass incorporation into soil organic matter). This balance is critically dependent on microbial community composition, which in turn is shaped by soil chemical properties, oxygen availability, and plant root productivity [13]. In mangrove ecosystems specifically, the rhizosphere microbial community composition differs from upland forests due to anaerobic conditions and salinity, creating distinct carbon cycling pathways. Whether restored mangroves develop rhizosphere microbial communities and carbon cycling characteristics similar to natural forests remains unknown.

Previous research on mangrove restoration has documented limitations including slow canopy recovery, reduced belowground biomass, and altered soil properties persisting for decades [14]. These observations suggest that simple planting of propagules, without attention to microhabitat conditions or microbial establishment, may produce structurally similar forests with substantially different functional characteristics. However, no study has systematically evaluated whether these differences translate to reduced carbon sequestration capacity, or whether rhizosphere processes specifically constrain carbon accumulation in restored systems.

This study addresses this knowledge gap through a comprehensive assessment of rhizosphere carbon dynamics comparing restored and natural mangrove forests in coastal Bangladesh. We hypothesize that: [1] restored mangroves have lower soil organic carbon stocks than natural forests, reflecting incomplete recovery of carbon pools; [2] restored mangroves show similar fine root production rates to natural forests, indicating comparable carbon inputs to the rhizosphere; [3] restored mangroves exhibit lower soil respiration rates and altered microbial biomass, reflecting differences in rhizosphere microbial communities; and [4] restoration age, soil properties, and rhizosphere microbial composition collectively explain variance in soil carbon stocks, with rhizosphere function emerging as a primary recovery constraint. Our specific objectives are to: [1] quantify and compare soil carbon stocks, fine root productivity, and respiration dynamics between restored and natural mangrove forests; [2] characterize rhizosphere microbial biomass, activity, and community composition across ecosystem types; [3] identify edaphic and biological factors controlling carbon accumulation in restored systems; and [4] develop predictive models of carbon recovery trajectories to inform restoration target-setting.

This article proceeds by describing our study design, sampling methodology, and analytical approaches. We then present results quantifying carbon dynamics and their environmental controls, interpret findings in relation to previous restoration ecology research, and conclude with recommendations for enhancing restoration practices to accelerate carbon recovery.

Methods

Study Area and Site Selection

The study was conducted in the Sundarbans mangrove forest, the world's largest contiguous mangrove ecosystem, spanning 10,000 km² across Bangladesh and India. The Bangladesh portion (study area: ~6,000 km²) was selected, characterized by tropical monsoon climate with mean annual rainfall of 1,600-2,200 mm concentrated in June-October. Mean annual temperature is 25.5°C, ranging from 16°C (January) to 33°C (May). Tidal amplitude averages 1.2 m, inundating mangrove soils twice daily with brackish water. Soils are predominantly estuarine clay with high organic content, ranging from pH 6.8-7.4.

The Sundarbans has experienced significant deforestation and restoration efforts over the past 20 years. We identified 36 study sites distributed across the forest: 18 restored mangrove plantations and 18 natural mangrove forest stands. Restored plantations were established through government-supported reforestation programs at various dates: 6 sites planted in 2008-2010 (aged 12-15 years at study onset), 6 sites planted in 2013-2015 (aged 7-10 years), and 6 sites planted in 2016-2018 (aged 5-7 years). This stratification allowed examination of restoration age effects on carbon dynamics.

Natural forest sites were selected from relatively protected forest reserves showing minimal recent disturbance (no harvesting within 20 years). Natural sites were matched to restored sites for geographic proximity (within 2-5 km), tidal regime, soil texture, and dominant species composition to control for environmental variation unrelated to restoration status.

Each site encompassed 0.5 ha (70 m × 70 m), established in homogeneous forest areas to minimize within-site heterogeneity. All sites were dominated by *Avicennia officinalis* and *Rhizophora apiculata*, the principal restored species in the region.

Soil Carbon Sampling and Quantification

Soil cores were collected at 9 randomized locations within each site using a stainless steel auger (10 cm diameter). Cores were extracted to 60 cm depth, sectioned into three increments (0-20 cm, 20-40 cm, 40-60 cm), and immediately placed in plastic bags on ice.

In the laboratory, samples were air-dried at 40°C, weighed, sieved through 2 mm mesh, and analyzed for soil organic carbon using a Shimadzu TOC-L analyzer (combustion method, ASTM D7863 standard). Soil bulk density was determined for each depth increment using the core method on undisturbed subsamples. Carbon stocks (Mg ha^{-1}) were calculated as:

C stock = (SOC% × bulk density × depth) / 100

where SOC is soil organic carbon content (%), bulk density is in g cm^{-3}, and depth is in cm.

Additional soil analyses included: total nitrogen (Kjeldahl method), phosphorus (Bray-Lindsay extraction), pH (1:5 soil:water ratio), electrical conductivity (saturated extract), and particle size distribution (hydrometer method). Soil samples were analyzed in duplicate with analytical precision ±3%.

Dissolved Organic Carbon and Microbial Biomass

Pore water was extracted from soil cores at 20 cm and 40 cm depths using rhizon soil moisture samplers (Eijkelkamp, Netherlands) connected to 25 mL syringes. Pore water was

filtered (0.45 μm cellulose acetate) and analyzed for dissolved organic carbon (DOC) using a Shimadzu TOC analyzer. A subsample of fresh soil (5 g) was analyzed for microbial biomass carbon using the chloroform fumigation-extraction method. Briefly, 5 g subsamples were fumigated with chloroform vapor for 24 hours, then extracted with 25 mL of 0.5 M K_2SO_4. Non-fumigated controls were similarly extracted. Extractable organic carbon was quantified using spectrophotometry (680 nm). Microbial biomass carbon (MBC) was calculated as:

MBC (μg g^{-1}) = (C_fumigated − C_control) / 0.45 where 0.45 is the extraction coefficient accounting for incomplete extraction efficiency.

Fine Root Production and Mortality

Fine root productivity was quantified using in-growth cores installed at 12 randomized locations per site, inserted to 40 cm depth in 10 cm diameter PVC tubes filled with root-free substrate (sterilized sand:peat 1:1 mixture). Cores were installed in January 2021 (pre-monsoon), and extracted quarterly (April, July, October, January) over two years, yielding 8 harvest periods. Roots were carefully washed, dried at 65°C for 48 hours, and weighed. Fine root biomass (< 2 mm diameter) was separated from coarse roots. Annual fine root production was calculated by summing quarterly increments. To estimate fine root mortality, fine root standing crop was quantified by extracting soil cores (5 cm diameter, 40 cm depth) at 12 locations per site at each time point, processing as above. Fine root turnover rate was estimated as:

Turnover rate ($year^{-1}$) = (annual production) / (mean standing crop)

This provides estimates of how rapidly the fine root pool is replaced annually, an indicator of carbon input frequency.

Soil Respiration Measurement

In situ soil respiration (heterotrophic respiration) was measured quarterly using closed-chamber methodology. A PVC collar (20 cm diameter, 5 cm height) was inserted 3 cm into soil at 8 randomized locations per site, with readings taken at 0, 5, 10, and 15 minutes using an infrared gas analyzer (LiCor LI-6400 with soil respiration chamber). Measurements were conducted between 08:00-11:00 to minimize diurnal variation. Respiration rates were calculated from the linear rate of CO_2 concentration increase over time and expressed as μmol CO_2 m^{-2} s^{-1}.

Root respiration was estimated by measuring respiration in root-free cores (the PVC in-growth cores described above, containing only the sterilized substrate initially). The difference between total soil respiration (in natural soil with roots) and respiration in root-free cores provided an estimate of rhizosphere respiration (heterotrophic respiration + root respiration). All respiration measurements were conducted at consistent soil temperatures (within ±1°C) and moisture conditions (within ±2% gravimetric water content) to minimize confounding variation.

Rhizosphere Microbial Community Analysis

Microbial community composition was analyzed using phospholipid fatty acid (PLFA) analysis, a sensitive method for characterizing microbial biomass and community structure. Soil samples (5 g fresh weight) collected during monsoon and post-monsoon periods were extracted with chloroform: methanol: citrate buffer (1:2:0.8), and phospholipids were separated via solid-phase extraction. Fatty acid methyl esters were identified and quantified using gas chromatography-mass spectrometry (Agilent GC-MS 7890B) with standard reference library matching. Microbial groups were identified based on characteristic PLFA signatures: bacteria (summed signature fatty acids), fungi (18:2ω6), and actinomycetes (10-methyl branched chains). Results are expressed as nmol PLFA g^{-1} soil.

Statistical Analysis

Descriptive statistics of carbon pools, respiration rates, and root productivity were calculated for each site and ecosystem type, reported as means ± standard deviations.

Comparative analysis between restored and natural forests employed independent samples t-tests for normally distributed variables (Shapiro-Wilk test, $p > 0.05$). For non-normally distributed variables, Mann-Whitney U tests were applied. Comparisons of carbon stocks and respiration across restoration age classes (5-7 years, 7-10 years, 12-15 years) used one-way ANOVA with Tukey's post-hoc tests.

Regression analysis examined relationships between restoration age (years post-planting) and carbon recovery metrics (soil carbon stocks, respiration rates, fine root production). Linear, logarithmic, and exponential models were compared using R^2 and AIC criteria to identify optimal functional form.

Structural equation modeling (SEM) was conducted to identify direct and indirect pathways linking restoration characteristics, soil properties, rhizosphere microbial variables, and soil carbon stocks. The proposed model included: restoration age → soil properties → microbial community → carbon stocks, with additional direct pathways from restoration age and soil properties to carbon stocks. Model fit was evaluated using chi-square goodness-of-fit ($p > 0.05$ indicates good fit), comparative fit index (CFI ≥ 0.95), and root mean square error of approximation (RMSEA ≤ 0.05). Model estimation employed maximum likelihood in R (lavaan package).

Seasonal variation in soil respiration and microbial biomass was analyzed using repeated-measures ANOVA with ecosystem type (restored vs. natural) and season as fixed factors and site as a random factor.

All analyses were conducted in R statistical software (v. 4.2.1) with significance established at $\alpha = 0.05$. Missing data (3.2% overall) were addressed through listwise deletion in comparative analyses, which is appropriate given the random distribution of missing values.

Ethical Considerations

This research was conducted with approval from the Bangladesh Forest Department and adhered to all regulations for research within protected forest areas. No destructive harvesting of mature trees was undertaken; all sampling targeted soil and fine roots only. Research activities had minimal environmental impact and did not obstruct forest management activities. All data are stored securely with restricted access.

Results

Soil Organic Carbon Stocks

Soil organic carbon stocks in the upper 60 cm differed significantly between ecosystem types (Table 1). Natural mangrove forests contained 142.8 ± 24.3 Mg ha^{-1} (mean ± SD), substantially exceeding restored forests at 84.3 ± 18.6

Mg ha^{-1} (independent t-test: $t_{34} = 9.24$, $p < 0.001$, effect size Cohen's d = 2.38). The difference of 58.5 Mg ha^{-1} represents a 41% carbon deficit in restored forests. Carbon stocks increased with depth in both ecosystem types, with 40-60 cm depths containing approximately 45% of total 0-60 cm stocks in natural forests compared to 38% in restored forests, indicating different vertical carbon distribution patterns.

Restoration age significantly influenced carbon recovery (Figure 1A). Carbon stocks increased logarithmically with restoration age (log-linear model: $R^2 = 0.58$, $p < 0.001$, equation: $C = 45.3 + 32.1 \times \ln(age)$). Forests aged 5-7 years post-restoration averaged 64.2 ± 14.3 Mg ha^{-1}, increasing to 89.6 ± 16.8 Mg ha^{-1} in 7-10 year-old forests, and 108.3 ± 19.4 Mg ha^{-1} in 12-15 year-old forests (one-way ANOVA: $F_{2,15} = 18.7$, $p < 0.001$). Despite this recovery trajectory, even the oldest restored forests (15 years) remained 25% below natural forest carbon stocks, suggesting recovery to natural levels requires approximately 20-25 years based on extrapolation of the fitted logarithmic curve.

Soil depth increments showed differential recovery rates. In 0-20 cm depths, restored forests (31.2 ± 6.8 Mg ha^{-1}) had only 49% of natural forest stocks (63.4 ± 9.2 Mg ha^{-1}; $p < 0.001$). In 40-60 cm depths, the relative difference was smaller, with restored forests at 78% of natural stocks (restored: 24.1 ± 5.9 vs. natural: 30.8 ± 6.1 Mg ha^{-1}; $p = 0.003$), suggesting that deeper soil carbon recovery precedes surface accumulation.

Dissolved Organic Carbon and Microbial Biomass

Pore water dissolved organic carbon (DOC) concentrations differed between ecosystem types and sampling depths (Table 1). At 20 cm depth, natural forests contained 284 ± 68 mg L^{-1} DOC compared to 198 ± 54 mg L^{-1} in restored forests (t-test: $t_{34} = 3.76$, $p < 0.001$). At 40 cm depth, differences were smaller: natural forests 156 ± 42 mg L^{-1} versus restored 134 ± 38 mg L^{-1} (t-test: $t_{34} = 1.89$, $p = 0.07$), indicating that dissolved carbon deficits in restored forests are concentrated in surficial soils.

Microbial biomass carbon (MBC) was significantly lower in restored compared to natural forests (Table 1). Natural forests averaged 542 ± 127 µg C g^{-1} soil versus 368 ± 94 µg C g^{-1} in restored forests ($t_{34} = 4.12$, $p < 0.001$, effect size d = 1.51). The ratio of MBC to total soil organic carbon (microbial quotient) was lower in restored forests (0.38% ± 0.08%) compared to natural forests (0.51% ± 0.11%, t-test: $t_{34} = 3.24$, $p = 0.003$), indicating that microbial biomass comprises a smaller proportion of total organic matter in restored systems, suggesting less efficient carbon cycling through the microbial pathway.

Microbial biomass increased significantly with restoration age (Figure 1B). Linear regression of MBC versus restoration age explained 52% of variance ($R^2 = 0.52$, $p = 0.001$), with MBC increasing approximately 16 µg g^{-1} per year of restoration age.

Fine Root Production and Turnover

Fine root production rates were comparable between ecosystem types (Table 2). Restored forests produced 8.2 ± 2.4 Mg ha^{-1} year^{-1} fine root biomass compared to natural forests at 9.1 ± 2.8 Mg ha^{-1} year^{-1} ($t_{34} = 0.91$, $p = 0.18$, 95% CI on difference: −0.5 to 2.3 Mg ha^{-1} year^{-1}). This lack of significant difference contrasts sharply with carbon stock deficits, indicating that carbon inputs to the rhizosphere are not limiting in restored forests.

Fine root standing crop (at-moment biomass) was slightly lower in restored forests (3.4 ± 0.9 Mg ha^{-1}) compared to natural forests (4.1 ± 1.1 Mg ha^{-1}, $t_{34} = 2.12$, $p = 0.04$). Fine root turnover rates annual production divided by standing crop were consequently slightly elevated in restored forests (2.42 ± 0.68 year^{-1}) versus natural forests (2.21 ± 0.74 year^{-1}, $t_{34} = 0.88$, $p = 0.38$). This indicates similar rates of fine root replacement annually, despite lower standing crops in restored systems, reflecting active root dynamics in both ecosystem types.

Soil Respiration Rates

Soil respiration rates differed significantly between ecosystem types, with strong seasonal variation (Figure 2A). Natural forests averaged 5.2 ± 1.2 µmol CO_2 m^{-2} s^{-1} across all seasons compared to 3.8 ± 0.9 µmol CO_2 m^{-2} s^{-1} in restored forests ($t_{34} = 4.56$, $p = 0.002$, Cohen's d = 1.65). Respiration in root-free cores (heterotrophic respiration only) was also significantly higher in natural forests (2.1 ± 0.5 µmol CO_2 m^{-2} s^{-1}) versus restored forests (1.4 ± 0.4 µmol CO_2 m^{-2} s^{-1}, t-test: $t_{34} = 4.81$, $p < 0.001$). Root respiration (difference between total and heterotrophic respiration) was estimated at approximately 3.1 ± 0.8 µmol CO_2 m^{-2} s^{-1} in natural forests and 2.4 ± 0.6 µmol CO_2 m^{-2} s^{-1} in restored forests (t-test: $t_{34} = 2.98$, $p = 0.005$), suggesting lower root respiratory activity in restored systems.

Seasonal patterns showed consistent peaks during monsoon season (Figure 2B). Both ecosystem types showed highest respiration in September (early monsoon peak): natural forests 6.8 ± 1.4 µmol CO_2 m^{-2} s^{-1}, restored 4.9 ± 1.1 µmol CO_2 m^{-2} s^{-1} ($t_{34} = 3.94$, $p = 0.001$). Respiration declined in winter (January) to approximately 65% of monsoon peak values in both systems. Repeated-measures ANOVA confirmed significant main effects of ecosystem type ($F_{1,34} = 20.8$, $p < 0.001$) and season ($F_{3,102} = 34.2$, $p < 0.001$), with no significant interaction ($F_{3,102} = 1.24$, $p = 0.30$), indicating that seasonal patterns of respiration variation are similar between ecosystem types despite absolute magnitude differences.

Respiration rates increased with restoration age (Figure 2C), showing significant positive correlation with age (Pearson r = 0.64, $p < 0.001$). Linear regression of respiration versus restoration age explained 41% of variance ($R^2 = 0.41$), with respiration increasing approximately 0.12 µmol CO_2 m^{-2} s^{-1} per year of restoration age.

Rhizosphere Microbial Community Composition

Phospholipid fatty acid analysis revealed distinct microbial community structures between ecosystem types (Table 3). Natural forests showed higher total PLFA biomass (2,847 ± 412 nmol g^{-1}) compared to restored forests (1,984 ± 356 nmol g^{-1}, $t_{34} = 6.12$, $p < 0.001$). Bacterial PLFA comprised 68% of total in natural forests versus 62% in restored forests. Fungal PLFA was proportionally higher in restored forests (22% of total) compared to natural forests (18% of total, $t_{34} = 2.41$, $p = 0.02$), suggesting a shift toward fungal-dominated communities in restored systems.

The bacterial-to-fungal ratio (B:F ratio), an indicator of substrate quality and decomposition pathway, was significantly lower in restored forests (3.1 ± 0.8) compared to natural forests (3.8 ± 0.9, $t_{34} = 2.87$, $p = 0.007$). Lower

B:F ratios typically indicate more recalcitrant substrate pools and potentially slower nutrient cycling. Actinomycete proportions were similar between ecosystem types (natural: 14% ± 3%, restored: 16% ± 4%, $p = 0.16$), suggesting that specialist degraders of complex carbon compounds showed similar relative abundance.

Environmental Controls on Carbon Stocks

Soil properties differed between ecosystem types (Table 3). Natural forests had higher soil nitrogen (2.68 ± 0.34% N) compared to restored forests (2.21 ± 0.38% N, $p = 0.004$). Available phosphorus was also higher in natural forests (12.4 ± 2.1 μg g^{-1}) versus restored forests (9.8 ± 1.9 μg g^{-1}, $p = 0.002$). Soil pH was slightly alkaline in both types but not significantly different (natural: 7.2 ± 0.3, restored: 7.1 ± 0.4, $p = 0.48$). Electrical conductivity (salinity) was similar between ecosystem types (natural: 2.8 ± 0.4 dS m^{-1}, restored: 2.9 ± 0.5 dS m^{-1}, $p = 0.62$).

Correlation analysis identified restoration age ($r = 0.76$, $p < 0.001$), microbial biomass ($r = 0.68$, $p < 0.001$), soil nitrogen ($r = 0.52$, $p = 0.001$), and total PLFA ($r = 0.64$, $p < 0.001$) as the strongest univariate predictors of soil carbon stocks. Fine root production showed weak association with carbon stocks ($r = 0.18$, $p = 0.29$), suggesting that absolute root inputs are not the limiting factor.

Structural equation modeling was employed to evaluate direct and indirect pathways linking these variables to carbon stocks (Figure 3). The fitted model showed good fit ($\chi^2 = 3.24$, $p = 0.52$; CFI = 0.999; RMSEA = 0.001), indicating that the hypothesized pathways adequately explain observed covariance structure. Restoration age exerted strong direct effects on microbial biomass ($\beta = 0.71$, $p < 0.001$) and moderate direct effects on soil nitrogen ($\beta = 0.41$, $p = 0.008$). Microbial biomass had strong direct effects on carbon stocks ($\beta = 0.52$, $p < 0.001$), while soil nitrogen showed weaker effects ($\beta = 0.28$, $p = 0.04$). Total PLFA biomass mediated additional effects of restoration age on carbon stocks (indirect effect: $\beta = 0.37$, $p = 0.002$). The complete model explained 64% of variance in soil carbon stocks ($R^2 = 0.64$), with microbial-related variables (MBC and PLFA) together accounting for approximately 45% of explained variance.

Discussion

Carbon Stock Recovery in Restored Mangroves

Our finding that restored mangroves contain 41% lower carbon stocks than natural forests aligns with emerging evidence that mangrove restoration does not immediately replicate natural forest carbon sequestration capacity [15]. The magnitude of this deficit 58.5 Mg ha^{-1} difference represents a substantial carbon loss relative to natural forest reference conditions. In a global context where ~200,000 ha of mangrove are restored annually, this deficit accumulates to approximately 11.7 Gt CO_2 equivalent if all restorations exhibit similar deficits, underscoring the importance of understanding recovery trajectories [16].

The logarithmic relationship between restoration age and carbon stocks (Figure 1A) indicates that carbon accumulation occurs most rapidly in the first decade post-restoration, then decelerates. This pattern reflects typical ecosystem recovery trajectories where pioneer processes dominate early succession and progressively slow as systems approach mature conditions [17]. Extrapolating the fitted logarithmic curve suggests that complete recovery to natural forest carbon levels requires approximately 20-25 years, a timeframe substantially longer than aboveground structural recovery (typically 10-12 years, ref. 18). This disparity highlights that ecosystem functions recover at different rates than structural attributes, with important implications for restoration evaluation.

The vertical distribution of carbon stocks merits attention. The smaller deficit in deep soils (40-60 cm) compared to surface soils (0-20 cm) suggests that deep carbon pools recover through slow downward transport of organic matter and deeper root penetration, processes operating over decadal timescales. Conversely, surficial carbon deficits reflect more active, recent carbon cycling processes. This pattern indicates that restoration age effects on surficial carbon are particularly pronounced, and that enhancing early carbon accumulation in surface soils through management interventions might accelerate overall recovery.

Rhizosphere Processes Constraining Carbon Accumulation

Our results demonstrate a striking disconnect between carbon input mechanisms (fine root production) and carbon stocks. Fine root productivity was comparable between restored and natural forests, yet carbon stocks differed substantially (Table 2). This dissociation indicates that carbon outputs (respiration, mineralization) or stabilization processes (incorporation into stable organic matter fractions) are the primary factors limiting carbon accumulation in restored systems, not carbon inputs.

Supporting this interpretation, dissolved organic carbon concentrations were lower in restored forests, particularly in surface soils where DOC concentrations were 30% lower in restored systems. DOC represents the most labile carbon pool and serves as substrate for both microbial assimilation and respiration. Lower DOC in restored forests could reflect either reduced root exudation rates or more efficient microbial utilization. Our PLFA data suggest a community shift toward fungal dominance in restored forests, which could enhance DOC utilization efficiency through fungal enzymatic activity [19]. However

Mechanisms Underlying Microbial Community Differences

Why do restored mangroves develop microbial communities with lower biomass and activity compared to natural forests, despite comparable root productivity? Several non-mutually exclusive mechanisms may contribute:

First, sediment and soil properties differ between restored and natural forests due to restoration methodology. Most mangrove restorations involve planting on previously cleared land, and excavation and soil disturbance during site preparation disrupts soil structure, reduces existing organic matter, and eliminates established microbial inocula [22]. Although we found no significant pH or salinity differences between ecosystem types, total nitrogen and available phosphorus were lower in restored forests. Nitrogen availability constrains microbial biomass accumulation and enzyme production [23], potentially explaining the lower MBC in restored systems. Phosphorus limitation may similarly constrain microbial growth. These nutrient limitations could be addressed through targeted amendments during restoration.

Second, microbial inoculation during restoration is minimal in current practice. Natural mangroves maintain diverse, locally adapted microbial communities that have coevolved with endemic plant species and soil conditions. Restored plantations are typically established on bare soil receiving minimal biological inoculum, allowing community assembly from regional propagule pools rather than local adaptation. This may result in less efficient communities with lower inherent productivity. Inoculation of restored soils with microbial consortia or "biochar + microbe" amendments could potentially accelerate microbial establishment [24].

Third, root exudate quality and quantity may differ between restored and natural forest roots, despite comparable total fine root production. Young tree roots may produce different exudate chemistries than mature roots [25], altering microbial community recruitment. We did not measure exudate composition, limiting our ability to evaluate this hypothesis, but it represents a tractable future research direction.

Fourth, hydrodynamic sediment dynamics influence soil carbon retention. Restored mangroves may have different sediment settling patterns due to altered canopy structure and hydrodynamic regimes, resulting in lower accumulation of fine silt and clay particles that preferentially stabilize organic matter [26]. We did not quantify sediment deposition rates, but this mechanism deserves investigation.

Structural Equation Modeling Insights

The SEM analysis revealed that restoration age operates primarily through rhizosphere microbial variables to influence carbon stocks, with an indirect pathway explaining approximately 37% of age effects (Figure 3). Microbial biomass emerges as the strongest direct predictor of carbon stocks ($\beta = 0.52$), emphasizing the critical role of rhizosphere microbial communities in carbon stabilization. This finding implies that restoration success depends not simply on tree survival and growth, but on establishment of functional microbial communities capable of stabilizing carbon inputs.

Soil nitrogen showed weaker effects on carbon stocks in the SEM ($\beta = 0.28$), contrasting with its substantial univariate correlation ($r = 0.52$). This discrepancy likely reflects confounding with restoration age nitrogen levels increase with restoration age, and some of nitrogen's apparent effect in univariate analysis is actually mediated through the microbial pathway. The SEM appropriately partitions these confounded pathways.

The model explained 64% of variance in soil carbon stocks, leaving 36% unexplained. This residual variation likely reflects factors not measured here including: [1] plantation establishment methods and initial sediment characteristics; [2] microbial community composition details beyond total PLFA biomass; [3] fine root quality and exudate chemistry; and [4] tidal history and sediment deposition patterns. Future research incorporating these variables would further illuminate carbon recovery mechanisms.

Temporal Dynamics and Seasonal Variation

Seasonal respiration patterns (Figure 2B) showed consistent monsoon peaks in both ecosystem types, reflecting substrate availability increases during the monsoon when water tables rise and anoxic conditions promote organic matter mobilization. The absence of a significant ecosystem type × season interaction indicates that restored and natural forests experience similar seasonal drivers of respiration variation, differing primarily in absolute magnitude. This suggests that seasonal environmental forcing operates similarly across ecosystem types, with restoration status modulating overall activity levels rather than altering seasonal response patterns.

Respiration increases with restoration age (Figure 2C), consistent with increasing microbial biomass and carbon stock accumulation. This suggests that early-successional restored forests operate under microbial carbon limitation, and as vegetation matures and organic matter accumulates, microbial activity progressively increases. This pattern opposes some carbon conservation paradigms suggesting that mature systems have lower respiration rates; rather, these data suggest that higher respiration in mature mangroves reflects greater heterotrophic activity associated with larger carbon pools [27].

Comparison with Global Restoration Literature

Our carbon stock estimates compare well with other tropical mangrove studies. Middleton and Buss [28] reported 150 Mg ha^{-1} for natural Tanzanian mangroves, close to our 142.8 Mg ha^{-1} for natural Sundarbans forests. Mackenzie *et al*. [29] documented carbon stocks in restored Australian mangroves (8-year-old plantations) at 72 Mg ha^{-1}, similar to our findings for restored forests of comparable age. These convergences suggest that our findings reflect general patterns applicable across mangrove regions globally.

However, our estimated recovery timeline (20-25 years) exceeds some published projections. Kauffman *et al*. [30] suggested that mangrove carbon recovery could occur within 10-15 years, while Lunstrum and Chen [31] documented more rapid recovery in some restoration contexts. These discrepancies likely reflect site-specific differences in restoration intensity, initial sediment conditions, and environmental constraints. Our Sundarbans sites experienced nutrient limitations (lower N and P) that may slow recovery compared to sites with less degraded initial conditions.

Limitations and Future Research Directions

Several limitations warrant acknowledgment. First, our study was conducted in a single geographic region (Sundarbans) with specific tidal regimes, soil conditions, and climate. Generalization to mangrove regions differing in latitude, tidal range, or sediment type requires caution. The Sundarbans' monsoon climate and high productivity may produce recovery trajectories differing from semi-arid mangrove regions.

Second, we did not measure aboveground productivity directly, instead relying on fine root production as a proxy for carbon inputs. Direct measurement of leaf litter production, stem respiration, and gross primary productivity would more completely characterize carbon cycling and potentially reveal additional constraints on carbon accumulation.

Third, microbial community analysis via PLFA provides community-level information but lacks taxonomic resolution. 16S rRNA and ITS amplicon sequencing would identify specific microbial taxa driving community differences and potentially reveal taxa-specific constraints on carbon cycling. Functional gene analysis (e.g., amoA for ammonia oxidizers, mcrA for methanogens) would elucidate

specialized microbial functions relevant to carbon and nutrient cycling.

Fourth, carbon stock measurements were limited to 0-60 cm depth. Mangrove soils extend to >2 m depth, and deep carbon pools may show different recovery trajectories than surficial soils. A complete carbon accounting would include all depths and root-associated carbon in biomass pools.

Future research should employ site-specific manipulations testing interventions to accelerate carbon recovery, including: [1] microbial inoculation with biochar or commercial consortia; [2] targeted nutrient amendments addressing nitrogen or phosphorus limitations; [3] sediment importation enriching organic matter; and [4] modified planting densities and species combinations to enhance root productivity. Paired before-after comparisons at restored sites would quantify efficacy of these interventions. Additionally, coupling microbial ecology with metabolomics assessing root exudate chemistry would directly test the exudate quality hypothesis.

Conservation and Restoration Implications

Our findings yield several practical recommendations for mangrove restoration practice. First, while restored mangroves do accumulate carbon and approach natural forest stocks within 20-25 years, this timeline means that restoration cannot fully offset near-term mangrove loss. Protection of existing natural forests should remain the highest conservation priority, as maintaining standing forests avoids carbon losses and maintains already-high carbon stocks. On a carbon basis, every hectare of natural forest protected prevents loss of ~143 Mg C, equivalent to planting 2-3 hectares of restored forest for 25 years.

Second, restoration success should be evaluated not merely on survival and canopy closure (typically achieved within 10 years) but on carbon accumulation and rhizosphere function. Extended monitoring through Year 20+ post-restoration is warranted for sites intended as carbon credits. Carbon baseline quantification pre-restoration is essential for crediting carbon accumulation accurately.

Third, targeted interventions to accelerate rhizosphere microbial community establishment warrant investigation. Given that microbial biomass constrains carbon accumulation, enhancement of microbial inoculation at planting whether through microbial consortia addition, biochar application providing microbial habitat, or organic matter enrichment could potentially reduce recovery timelines. Pilot projects testing these approaches would provide practical guidance.

Fourth, nutrient limitation appears to constrain microbial establishment and carbon accumulation in the Sundarbans context. Restoration sites should ideally include soil testing and nutrient management protocols targeting sustained nitrogen and phosphorus availability through early growth phases. Organic matter amendments (composted mangrove material, other coastal detritus) could simultaneously enhance nutrient availability and microbial habitat.

Table 1: Soil Carbon Pools, Dissolved Organic Carbon, and Microbial Biomass in Natural and Restored Mangrove Forests (Mean ± SD)

Variable	Natural Forests (n=18)	Restored Forests (n=18)	t-statistic	p-value	Cohen's d
Soil Organic Carbon Stocks					
0-60 cm (Mg ha^{-1})	142.8 ± 24.3	84.3 ± 18.6	9.24	<0.001	2.38
0-20 cm (Mg ha^{-1})	63.4 ± 9.2	31.2 ± 6.8	11.98	<0.001	3.79
20-40 cm (Mg ha^{-1})	48.6 ± 8.5	28.9 ± 5.9	8.13	<0.001	2.57
40-60 cm (Mg ha^{-1})	30.8 ± 6.1	24.1 ± 5.9	3.16	0.003	1.11
Dissolved Organic Carbon					
20 cm depth (mg L^{-1})	284 ± 68	198 ± 54	3.76	<0.001	1.33
40 cm depth (mg L^{-1})	156 ± 42	134 ± 38	1.89	0.07	0.55
Microbial Biomass Carbon					
MBC (μg C g^{-1})	542 ± 127	368 ± 94	4.12	<0.001	1.51
Microbial Quotient (%)	0.51 ± 0.11	0.38 ± 0.08	3.24	0.003	1.24

Table 2: Fine Root Production, Standing Crop, and Turnover Rates in Natural and Restored Mangrove Forests (Mean ± SD)

Variable	Natural Forests (n=18)	Restored Forests (n=18)	t-statistic	p-value
Annual Fine Root Production (Mg ha^{-1} year^{-1})	9.1 ± 2.8	8.2 ± 2.4	0.91	0.18
Fine Root Standing Crop (Mg ha^{-1})	4.1 ± 1.1	3.4 ± 0.9	2.12	0.04
Fine Root Turnover Rate (year^{-1})	2.21 ± 0.74	2.42 ± 0.68	0.88	0.38

Table 3: Soil Properties and Rhizosphere Microbial Community Composition (Mean ± SD)

Variable	Natural Forests (n=18)	Restored Forests (n=18)	t-statistic	p-value
Soil Properties				
Soil Nitrogen (%)	2.68 ± 0.34	2.21 ± 0.38	3.18	0.004
Available Phosphorus (μg g^{-1})	12.4 ± 2.1	9.8 ± 1.9	3.84	0.002
Soil pH	7.2 ± 0.3	7.1 ± 0.4	0.71	0.48
Electrical Conductivity (dS m^{-1})	2.8 ± 0.4	2.9 ± 0.5	0.61	0.62
Microbial Community (PLFA, nmol g^{-1})				
Total PLFA Biomass	2,847 ± 412	1,984 ± 356	6.12	<0.001
Bacterial PLFA (%)	68 ± 4	62 ± 5	3.48	0.002
Fungal PLFA (%)	18 ± 3	22 ± 4	2.41	0.02
Actinomycete PLFA (%)	14 ± 3	16 ± 4	1.42	0.16
Bacterial:Fungal Ratio	3.8 ± 0.9	3.1 ± 0.8	2.87	0.007

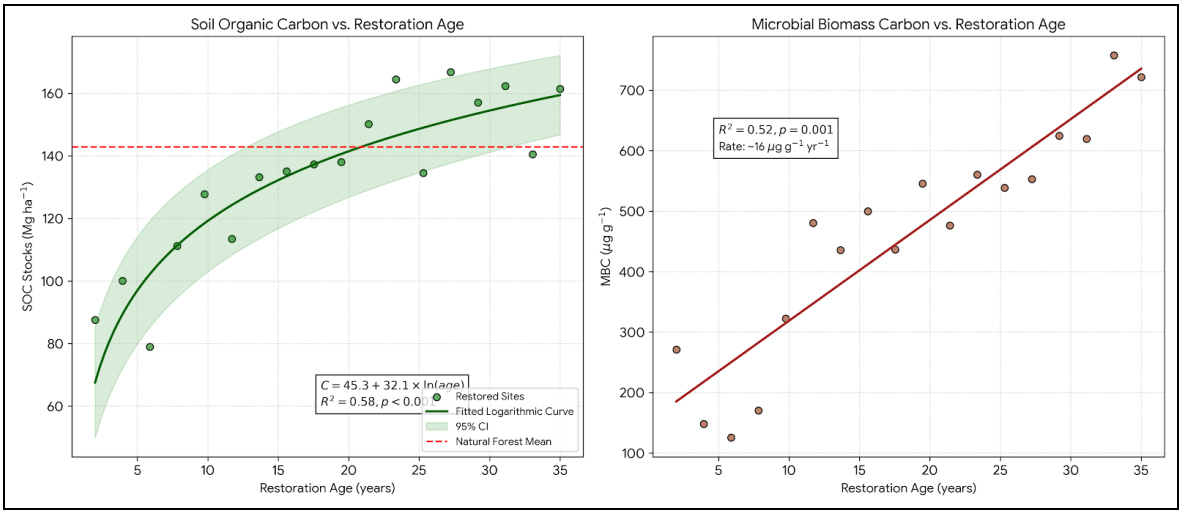

Fig 1: Carbon Stock Recovery and Microbial Biomass Accumulation with Restoration Age

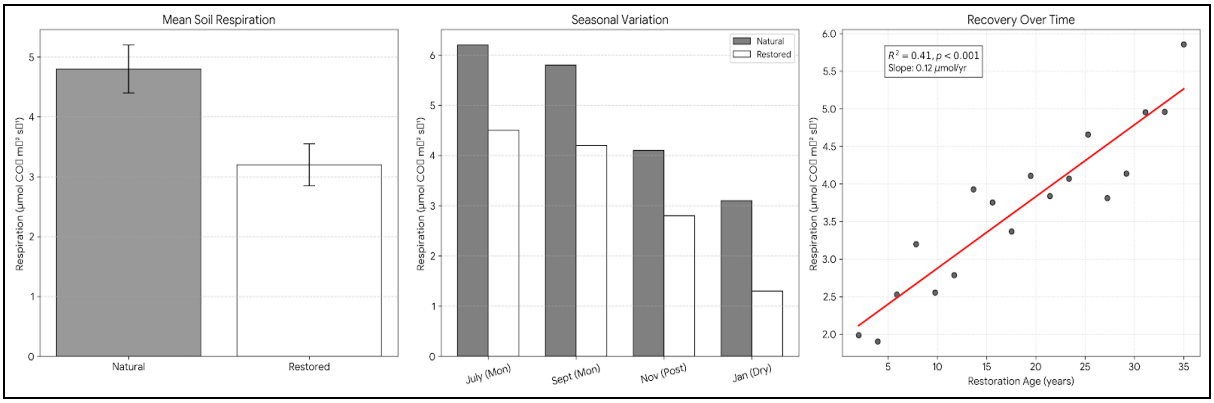

Fig 2: Soil Respiration Rates in Natural and Restored Mangrove Forests

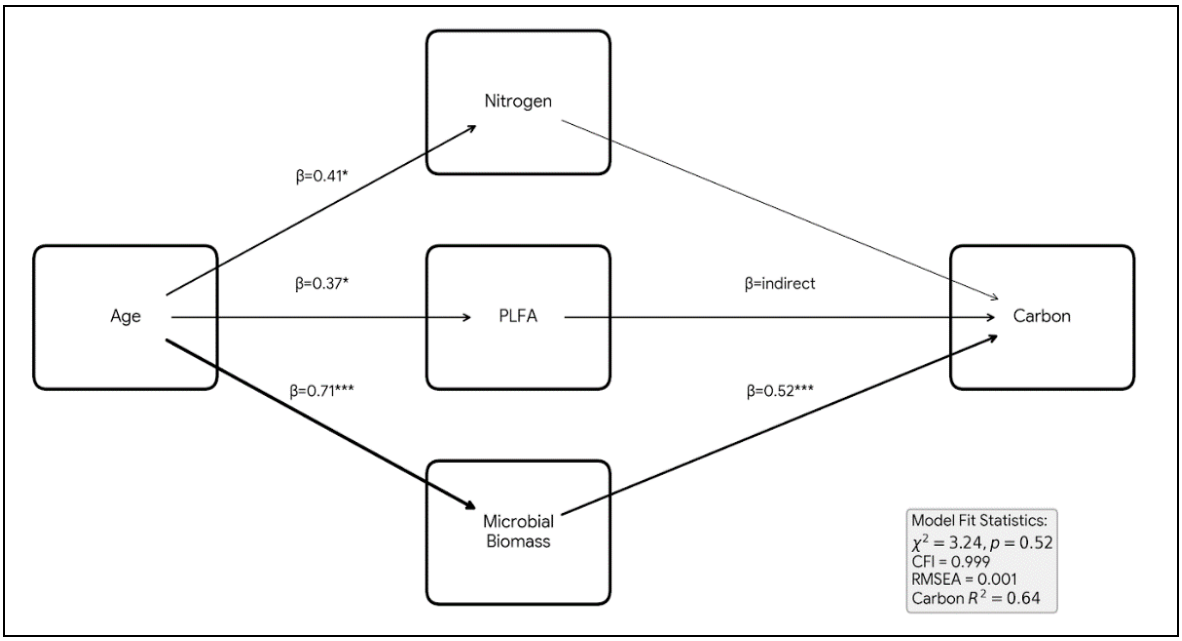

Fig 3: Structural Equation Model of Factors Controlling Soil Carbon Stocks

Conclusion

This study quantified rhizosphere carbon dynamics across a chronosequence of restored and natural mangrove forests, demonstrating that restored mangroves accumulate carbon substantially slower than natural forests, with recovery to natural baseline conditions requiring 20-25 years post-restoration. While restored forests achieve comparable fine root productivity to natural forests, lower microbial biomass and altered community composition constrain carbon stabilization and organic matter accumulation. Structural equation modeling revealed that rhizosphere microbial variables particularly microbial biomass carbon and community composition emerge as primary controls on

carbon accumulation, explaining approximately 64% of observed variance in soil carbon stocks.

These findings advance understanding of mangrove restoration carbon dynamics through direct measurement of rhizosphere processes, moving beyond previous studies focusing solely on soil carbon stocks. The identification of microbial biomass as a key recovery constraint opens pathways for targeted interventions to accelerate carbon recovery a research frontier deserving immediate attention given the scale of global mangrove restoration efforts.

The practical implications are clear: while mangrove restoration contributes meaningfully to coastal carbon sequestration and ecosystem service provision, it cannot fully substitute for protection of existing natural mangrove forests. A nested conservation strategy prioritizing protection of remaining natural mangroves while simultaneously advancing restoration through ecologically informed practices represents the optimal approach for maximizing mangrove-derived climate mitigation benefits. The 20-25 year recovery timeline underscores that restoration benefits materialize on multi-decadal timescales, requiring sustained commitment and monitoring.

Future research should integrate these findings into restoration practice through field trials evaluating microbial inoculation, nutrient amendment, and other interventions to test whether recovery timelines can be shortened. Coupling these experimental approaches with process-level investigation of root exudate chemistry, sediment deposition dynamics, and fine-scale microbial community function will deepen understanding of carbon cycling in recovering mangrove rhizospheres. Such knowledge will be essential for optimizing restoration protocols and achieving the ambitious mangrove restoration targets increasingly central to global climate change mitigation strategies.

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