



RESEARCH ARTICLE

Carbon pool management using organic sources for climate change mitigation in a rice-rice cropping system

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Abstract

The study examined carbon dynamics and physical fractionation patterns of short-term organic amendment incorporation along with mineral nitrogen (N) fertilisation for two consecutive seasons in a sandy clay loam soil (ultisol) under intensive rice cultivation. Four organic amendments plus an unamended control, with four N levels at 0, 35, 70 and 105 kg ha⁻¹ (designated N0, N35, N70, N105) were evaluated. The organic amendments included farmyard manure (FYM), jack tree (*Artocarpus heterophyllus* Lam.) leaves (ART), daincha (*Sesbania aculeata* Pers.) leaves (DNC) and rice husk biochar (RHB). Soil samples collected from 0–15 and 15–30 cm soil depths were analysed at harvest in both cropping seasons to evaluate treatment effects. Organic amendment addition substantially influenced total soil organic carbon (SOC) and its partitioning across physical fractions. Coarse particulate organic carbon (cPOC) was significantly higher and reached maximum concentrations (23.6 g kg⁻¹) under RHB combined with 105 kg N ha⁻¹. All the organic treatments showed more than 1 % increase in cPOC regardless of N supplementation. Fine particulate organic carbon (fPOC) peaked in both virippu and mundakan seasons when *Artocarpus* residues were paired with the high N rates. Light fraction organic carbon (LFOC) and intra-aggregate light fraction carbon concentrations were greatest under RHB without N addition, a pattern consistent across both seasons. Mineral-associated organic carbon (MinOC) accumulated maximum with RHB at 70 kg N ha⁻¹, but the treatments were found non-significant. Results indicate that LFOC exhibited the highest management sensitivity, followed by sand-sized heavy fraction organic carbon (HFOC) and silt-clay MinOC, corresponding to active, slow and passive SOC pools respectively. Hence, the integrated application of RHB with mineral N elevated SOC, suggesting this strategy could sustain productivity in laterite-based rice systems.

Keywords: biochar; carbon density fractions; carbon pools; carbon size fractions; laterite soil; organic manures; size

Introduction

Labile and recalcitrant carbon fractions fundamentally influence soil sequestration capacity. Long-term fertiliser trials have extensively documented nutrient provisioning and carbon storage benefits from diverse management strategies. However, comparative assessments examining total carbon budgets, including shifts in active and passive pool distributions over shorter timeframes, remain limited. Biochar amendments have enhanced physical, chemical and biological characteristics of highly weathered substrates (1). Management interventions affect both total organic carbon (TOC) stocks and fractional distributions in cultivated soils (2). In short-term assessments, TOC alone may lack sufficient sensitivity to detect management-induced quality changes over brief intervals. The contribution from particulate organic matter (POM) to A-horizon soil organic carbon (SOC) is estimated to be approximately 15 % in cropland, 14 % in grassland and 52 % in forested systems (3).

Organic mulching enhances soil moisture retention and canopy development, evidenced by increased biomass accumulation, root proliferation, leaf area expansion and grain production, which collectively improve water and nitrogen (N)

acquisition efficiency (4). *In situ* green manure incorporation alongside vermicompost and poultry manure reduced soil electrical conductivity and pH while elevating organic carbon relative to alternative treatment combinations (5). Extensive research has characterised how carbon restitution via wheat straw, farmyard manure, green manure and rice residues influences SOC stocks, grain yields, physical properties, organic matter content, total N and plant development (6–9). Particulate organic matter comprises an active soil organic matter (SOM) pool containing partially decomposed plant and animal residues, root fragments, fungal hyphae, spores, faecal pellets, faunal skeletons and seeds (10). This fraction represents 18–35 % of SOC, varying with agricultural management (11, 12). Current understanding about organic matter cycling in intensive agricultural systems is based primarily on net organic input quantity. Consequently, residue-based management studies often neglect linkages between carbon dynamics, input quality and biological responses, especially in double-cropped rice systems. Hence, in this line, this research was conducted. The study quantified carbon additions, their allocation across labile and stable fractions and relationships among the parameters, such as organic matter characteristics and net inputs during short-term rice-rice cultivation.

Materials and Methods

Experimental site

Field trials were established on farmland adjacent to Kerala Agricultural University (KAU), Thrissur, India (10°31'49" N, 76°12'53" E; 10 m elevation) between September 2015 and February 2016. Regional precipitation averages 3000 mm annually, with 70–75 % delivered by the Southwest Monsoon during the virippu season (June–September), 15–30 % from the Northeast Monsoon coinciding with mundakan cultivation (October–January) and residual showers during summer. During 2015–16, air temperature ranged from 35.5 °C (April–May 2015) to 19.9 °C (December–January 2016). Relative humidity fluctuated between 75–80 % in summer and peaked near 97 % in July. Pan evaporation reached 159.40 mm in January versus 77.00 mm in June. Experimental soil was classified as sandy clay loam (Ultisol), taxonomic class-fine loamy mixed isohyperthermic aquic ustifluvents; exhibiting pH 4.66, available nitrogen (N) 401.76 kg ha⁻¹ and 1.05 % organic carbon. Rice husk biochar (RHB) was generated through pyrolysis at 350–400 °C from locally sourced material. Physicochemical properties of organic amendments used, farmyard manure (FYM), jack tree (*Artocarpus heterophyllus* Lam.) leaves (ART), daincha (*Sesbania aculeata* Pers.) leaves (DNC) and rice husk biochar (RHB) are given in Table 1.

Table 1. Physicochemical properties of the soils of the experimental site

Property	Surface	Sub-surface
	0–15 cm	15–30 cm
pH	4.66	4.68
EC (dS m ⁻¹)	0.058	0.044
CEC (cmol (p +) kg ⁻¹)	6.02	6.00
Bulk density (Mg m ⁻³)	1.36	1.38
WHC (%)	48.47	48.03
OC (%)	1.05	1.00
Available N (kg ha ⁻¹)	401.76	360.43
Available P (kg ha ⁻¹)	18.46	17.74
Available K (kg ha ⁻¹)	87.34	84.14
Available Ca (mg kg ⁻¹)	300.42	268.36
Available Mg (mg kg ⁻¹)	31.25	26.56
Available S (mg kg ⁻¹)	5.89	5.04
Available Fe (mg kg ⁻¹)	235.73	213.45
Available Mn (mg kg ⁻¹)	5.43	4.36
Available Zn (mg kg ⁻¹)	2.84	2.44
Available Cu (mg kg ⁻¹)	6.87	6.39
Available B (mg kg ⁻¹)	0.27	0.23
MBC (mg kg ⁻¹)	153.30	131.27
Phosphatase (µg PNP h ⁻¹ g ⁻¹)	59.17	51.23
Dehydrogenase (µg TPF day ⁻¹ kg ⁻¹)	60.34	54.05
Texture	Sandy clay loam	

Experimental design

A randomized complete block design (RCBD) with 3 replications in 5.0 × 4.0 m plots evaluated a combination of four organic amendments, FYM, jack tree (*A. heterophyllus*) leaves (ART), daincha (*S. aculeata*) leaves (DNC) and rice husk biochar (RHB) in combination with mineral urea at 0, 35, 70 and 105 kg ha⁻¹ (designated N₀, N₃₅, N₇₀, N₁₀₅). Thus, the total of 20 treatments were evaluated (Table 2). Eighteen-day-old 'Jyothi' rice seedlings were transplanted at 15 × 10 cm spacing. The major nutrients of N, P and K were supplied as per POP, KAU 2016.

Table 2. Treatment particulars

Treatments	Details of treatments
T ₁	Non organic manure + 0 kg N ha ⁻¹
T ₂	Non organic manure + 35 kg N ha ⁻¹
T ₃	Non organic manure + 70 kg N ha ⁻¹
T ₄	Non organic manure + 105 kg N ha ⁻¹
T ₅	Farmyard manure at 5 t ha ⁻¹ + 0 kg N ha ⁻¹
T ₆	Farmyard manure at 5 t ha ⁻¹ + 35 kg N ha ⁻¹
T ₇	Farmyard manure at 5 t ha ⁻¹ + 70 kg N ha ⁻¹
T ₈	Farmyard manure at 5 t ha ⁻¹ + 105 kg N ha ⁻¹
T ₉	<i>Artocarpus</i> sp. at 5 t ha ⁻¹ + 0 kg N ha ⁻¹
T ₁₀	<i>Artocarpus</i> sp. at 5 t ha ⁻¹ + 35 kg N ha ⁻¹
T ₁₁	<i>Artocarpus</i> sp. at 5 t ha ⁻¹ + 70 kg N ha ⁻¹
T ₁₂	<i>Artocarpus</i> sp. at 5 t ha ⁻¹ + 105 kg N ha ⁻¹
T ₁₃	Daincha at 5 t ha ⁻¹ + 0 kg N ha ⁻¹
T ₁₄	Daincha at 5 t ha ⁻¹ + 35 kg N ha ⁻¹
T ₁₅	Daincha at 5 t ha ⁻¹ + 70 kg N ha ⁻¹
T ₁₆	Daincha at 5 t ha ⁻¹ + 105 kg N ha ⁻¹
T ₁₇	Rice husk biochar at 5 t ha ⁻¹ + 0 kg N ha ⁻¹
T ₁₈	Rice husk biochar at 5 t ha ⁻¹ + 35 kg N ha ⁻¹
T ₁₉	Rice husk biochar at 5 t ha ⁻¹ + 70 kg N ha ⁻¹
T ₂₀	Rice husk biochar at 5 t ha ⁻¹ + 105 kg N ha ⁻¹

Soil sampling and analysis

Post-harvest samples were collected from 0–15 and 15–30 cm depths, passed through 2 mm sieves (discarding plant debris) and analysed for total carbon via dry combustion (CHNS Elemental Analyser, Elementar Vario EL III). Inorganic carbon, determined by dilute HCl extraction (13), averaged 0.01 % and was subtracted from total carbon to calculate SOC. Bulk density was measured using 7.5 cm diameter × 7.5 cm length metal cores dried at 105 °C for 24 hr, calculated as oven-dry mass per core volume.

Size fractionation

An amount of 50 g of soil was dispersed in 150 mL of sodium hexametaphosphate (0.5 %) for 15 hr on a reciprocating shaker and suspensions were wet-sieved through 250 and 53 µm meshes (12, 14). Residue retained on 250 µm (coarse POM plus 250–2000 µm sand) and 53 µm sieves (fine POM plus 53–250 µm sand) were oven-dried at 50 °C and analysed for carbon concentration.

Density fractionation

Free light fraction organic matter (LFOM) was extracted using standard procedures (15). An amount of 25 g of soil was suspended in 40 mL of sodium iodide solution (specific gravity 1.72) and shaken for 30 min. After 48 hr equilibration, the floating material was aspirated, filtered, washed sequentially with 0.01 M CaCl₂ and distilled water and then dried at 50 °C. Settled material underwent 16 hr shaking followed by 48 hr equilibration to isolate intra-aggregate light fraction (iLFOM), processed identically to LFOM. The remaining heavy fraction was wet-sieved (53 µm), with retained material designated heavy fraction organic matter (HFOM). Silt-clay slurry passing 53 µm was centrifuged, dried and classified as mineral-associated organic matter (MinOM). All fractions (cPOM, fPOM, LFOM, iLFOM, HFOM, MinOM) were ground and analysed for carbon concentration by a CHNS analyser, yielding corresponding carbon pools (cPOC, fPOC, LFOC, iLFOC, HFOC, MinOC).

Statistical analysis

Treatment effects were evaluated through analysis of variance (16). Significant mean separations were determined using least significant difference tests at the 5 % probability level ($p < 0.05$). Correlation and regression analyses were performed using SPSS statistical software.

Results

Total carbon

Surface SOC reached 4.04 % under FYM (Table 3) without N supplementation during the virippu season, representing the maximum value, while RHB alone achieved 3.45 %. During mundakan, RHB-amended treatments consistently elevated carbon concentrations, with the sole RHB application yielding the highest level at 4.06 %. Subsurface horizons exhibited comparable patterns (Table 4), where unamended FYM produced a peak carbon content of 3.98 %. Rice husk biochar applied independently recorded 3.91 and 3.17 % during the virippu and mundakan seasons respectively.

Size fractions of organic carbon

Particulate organic carbon

Organic amendment incorporation significantly elevated cPOC concentrations relative to mineral N fertilisation alone ($p < 0.05$) (Table 5). Within surface horizons, RHB demonstrated greater influence on cPOC accumulation compared to FYM.

Coarse particulate organic carbon (cPOC)

Data in Table 5 indicate elevated carbon fraction concentrations during virippu relative to mundakan. Surface soil cPOC peaked at

23.6 g kg⁻¹ under RHB combined with 105 kg N ha⁻¹. All organic amendments, irrespective of N rate, exceeded 10 g kg⁻¹. This pattern persisted in mundakan, where RHB at the same N level achieved maximum cPOC of 20.70 g kg⁻¹. Subsurface cPOC distribution mirrored surface trends except in unamended treatments (Table 6). The control without fertiliser or organic inputs registered the highest subsurface concentration (24.50 g kg⁻¹) during virippu, a statistically significant value. During mundakan, this treatment maintained peak cPOC at 24.70 g kg⁻¹.

Fine particulate organic carbon (fPOC)

The data in Table 7 demonstrates elevated surface fPOC during the second season, with maximum concentrations observed under ART plus 105 kg N ha⁻¹ (5.80 and 6.30 g kg⁻¹ for virippu and mundakan, respectively), though differences among treatments were non-significant. Subsurface fPOC (Table 8) exhibited similar seasonal trends except in unamended plots, where concentrations declined. Farmyard manure combined with 105 kg N ha⁻¹ produced significantly the highest subsurface fPOC (14.00 g kg⁻¹). Within individual organic amendments, N rate variation consistently yielded fPOC exceeding 10.00 g kg⁻¹ (1.00 %), whereas remaining treatments recorded lower values. During mundakan, concentrations decreased slightly relative to virippu, peaking at 5.60 g kg⁻¹ under DNC with 35 kg N ha⁻¹.

Table 3. Effect of different organic sources on the total carbon of surface

Total carbon (%)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	2.21	4.04	2.90	2.32	3.45	1.74	2.97	1.76	1.81	4.06
N ₁	2.27	2.57	2.87	2.23	2.90	1.74	2.32	2.32	2.32	2.90
N ₂	2.32	2.56	2.27	2.21	2.32	1.73	2.32	1.74	1.91	2.57
N ₃	1.74	2.54	2.22	2.48	2.26	1.72	2.27	1.79	1.88	2.33
CD (0.05)	A - 0.007; B - 0.011; C - 0.010; A × B - 0.019; A × C - 0.014; B × C - 0.028; A × B × C - 0.039									

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 4. Effect of different organic sources on total carbon of sub-surface soil

Total carbon (%)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	2.08	3.98	2.26	2.24	3.91	1.11	2.87	2.27	1.74	3.17
N ₁	2.24	2.53	2.20	2.14	2.87	1.70	2.83	1.69	1.73	2.70
N ₂	2.16	2.51	2.20	2.10	2.28	1.11	2.28	1.71	1.73	2.38
N ₃	1.71	2.50	2.13	2.07	2.12	1.54	2.24	1.80	1.70	2.20
CD(0.05)	A - 0.009; B - 0.014; C - 0.012; A × B - 0.030; A × C - 0.018; B × C - 0.042; B × C - 0.059									

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 5. Effect of different organic sources on coarse particulate organic carbon of surface soil

Coarse particulate organic carbon (g kg ⁻¹)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	3.40	4.70	8.00	3.10	14.60	3.30	4.50	7.50	2.90	13.50
N ₁	4.50	2.90	5.90	3.20	16.40	4.40	2.50	4.30	2.60	14.30
N ₂	3.90	1.30	6.50	2.30	18.90	3.80	1.10	4.20	2.40	15.40
N ₃	1.60	3.80	6.80	2.50	23.60	1.50	3.50	3.60	2.40	20.70
CD (0.05)	A - 0.004; B - 0.007; C - 0.006; A × B - 0.010; A × C - 0.009; B × C - 0.013; A × B × C - 0.019									

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 6. Effect of different organic sources on coarse particulate organic carbon of sub-surface soil

Coarse particulate organic carbon (g kg ⁻¹)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	24.50	1.70	2.10	1.10	3.40	24.70	1.50	1.80	1.00	3.80
N ₁	23.30	0.90	1.80	6.60	1.40	23.50	0.60	1.60	6.40	1.20
N ₂	3.90	2.90	2.40	4.60	2.80	5.30	2.70	2.20	4.40	2.60
N ₃	3.50	1.40	1.80	1.50	3.40	3.70	1.10	1.70	1.30	3.10
CD (0.05) -	A - NS; B -0.009; C -0.008; A × B - 0.013; A × C - 0.012; B × C - 0.018;					A × B × C - 0.026				

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 7. Effect of different organic sources on fine particulate organic carbon of surface soil

Fine particulate organic carbon (g kg ⁻¹)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	3.80	5.00	4.30	5.40	2.60	3.90	5.20	4.50	3.80	5.00
N ₁	3.30	4.10	5.10	4.00	2.90	3.60	4.50	5.30	3.30	4.10
N ₂	4.80	3.30	5.60	3.50	2.80	3.90	3.60	5.90	4.80	3.30
N ₃	3.90	3.80	5.80	4.00	2.20	4.00	3.90	6.30	3.90	3.80
CD(0.05) -	A - 0.010; B - 0.017; C - 0.015; A × B-NS; A × C - NS; B × C -0.033; A × B × C -NS									

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 8. Effect of different organic sources on fine particulate organic carbon of sub-surface soil

Fine particulate organic carbon (g kg ⁻¹)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	4.90	2.40	3.10	3.80	3.50	4.70	2.60	3.50	4.20	3.50
N ₁	4.70	11.20	4.20	5.30	3.40	4.40	1.60	4.10	5.60	3.60
N ₂	4.80	13.00	5.50	4.50	3.70	4.40	2.80	5.40	5.00	3.80
N ₃	3.30	14.00	2.80	4.30	3.50	3.10	3.00	2.70	4.70	3.80
CD(0.05) A - B - C -	A × B - 0.008; B × C - 0.012; A × B × C - 0.017									

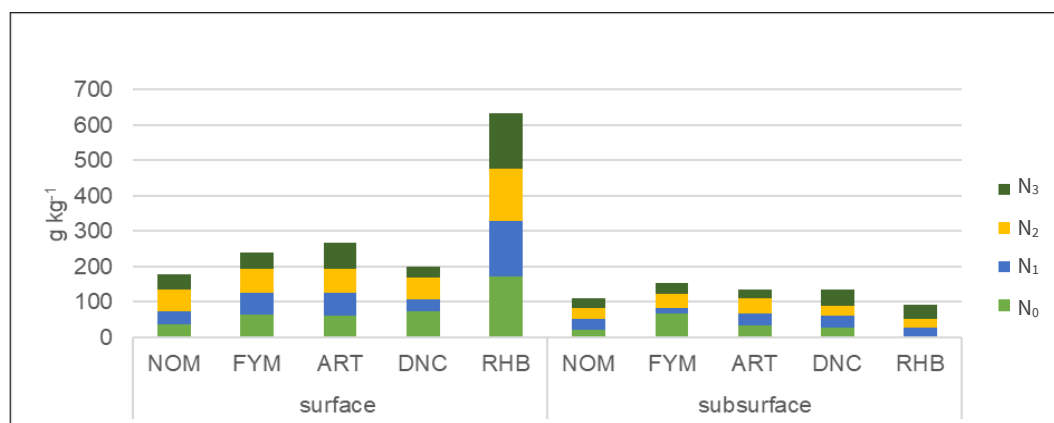
A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Density fractions

Density-based carbon fractions responded significantly to seasonal variation and amendment type. Concentrations increased during mundakan across all fractions except the heavy fraction, where changes were negligible. Surface horizons consistently contained higher carbon levels than subsurface layers for all fractions. Unamended treatments uniquely elevated subsurface mineral-associated organic carbon.

Light fraction organic carbon (LFOC)

Fig. 1 reveals that surface LFOC peaked significantly under RHB without N addition (171.30 g kg⁻¹). Generally, RHB treatments maintained LFOC exceeding 100.00 g kg⁻¹, surpassing alternative amendments. This pattern continued in mundakan, where RHB alone achieved a maximum LFOC of 174.00 g kg⁻¹. Subsurface LFOC reached significantly the highest concentrations under unamended FYM, recording 66.10 and 66.40 g kg⁻¹ during virippu and mundakan, respectively.

**Fig. 1.** Effect of different organic sources on light fraction organic carbon of soil-virippu.

NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Intra light fraction organic carbon (iLFOC)

As in the case of LFOC, the iLFOC values of surface soil reached maximum concentrations under RHB without N supplementation, recording 143.00 and 147.00 g kg⁻¹ during virippu and mundakan, respectively (Fig. 2). Rice husk biochar treatments consistently elevated iLFOC, though treatment interactions were non-significant. Subsurface iLFOC peaked under RHB alone during virippu (75.00 g kg⁻¹), followed by ART with 70 kg N ha⁻¹ (64.10 g kg⁻¹). In mundakan, RHB without N again yielded the highest subsurface iLFOC (75.30 g kg⁻¹). Treatment effects were statistically non-significant.

Heavy fraction organic carbon (HFOC)

Surface HFOC data appear in Table 9. The unamended control exhibited significantly maximum concentrations of 49.50 and 49.30 g kg⁻¹ during virippu and mundakan, respectively. Subsurface HFOC (Table 10) peaked under RHB without N at 38.10 g kg⁻¹ (virippu) and 38.00 g kg⁻¹ (mundakan), though treatment differences were non-significant.

Mineral-associated organic carbon (MinOC)

The surface soil MinOC ranged from 0.20 to 1.40 g kg⁻¹ in virippu (Table 11) and 0.30 to 1.70 g kg⁻¹ in mundakan seasons respectively. The treatments were found to be non-significant. The highest value (Table 12) of sub-surface soil MinOC was observed in the treatment daincha with 105 kg N ha⁻¹ (1.30 g kg⁻¹) during virippu and 1.40 g kg⁻¹ during mundakan seasons. The treatments were non significant with each other.

Amendment additions substantially influenced all pools, although N fertilisation significantly affected all fractions. Surface organic carbon fractions ranked by concentration as: LFOC (36.2–170 %) > iLFOC (20.4–143 %) > HFOC (22–49.5 %) > MinOC (0.2–1.7 %). Organic amendments significantly enhanced LFOC and iLFOC concentrations relative to mineral N ($p < 0.05$) (Table 5). Rice husk biochar exerted modest effects on MinOC while increasing LFOC and iLFOC by 66 % and 104 % over mineral N. Season × organic source interactions for HFOC were non-significant.

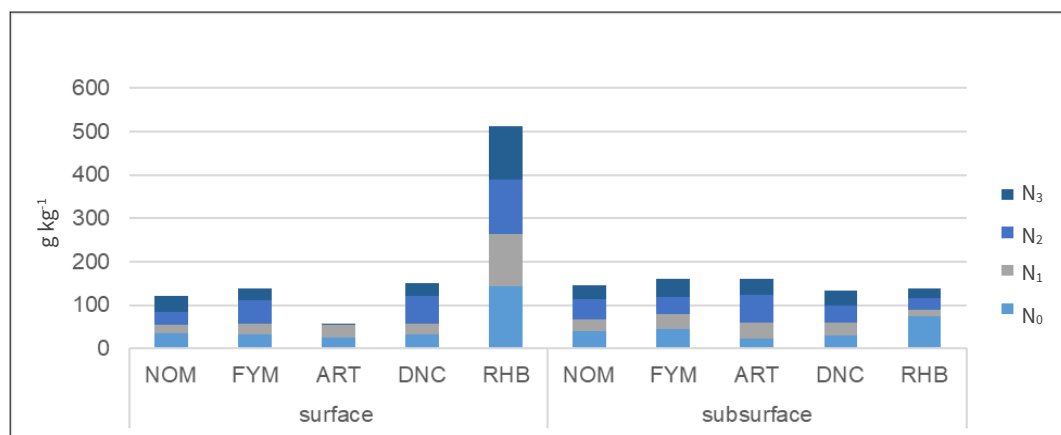


Fig. 2. Effect of different organic sources on intra light fraction organic carbon of soil-virippu.

NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 9. Effect of different organic sources on heavy fraction organic carbon of surface soil

Heavy fraction organic carbon of soil (g kg ⁻¹)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	49.50	21.60	23.00	24.80	30.50	49.30	21.50	22.80	24.50	29.00
N ₁	25.10	26.80	20.10	26.40	28.70	24.70	26.50	18.00	26.00	28.30
N ₂	24.80	22.10	23.90	27.50	24.90	24.60	21.80	23.50	26.70	24.50
N ₃	22.90	24.60	25.10	25.40	24.70	21.80	24.30	24.50	25.00	24.30
CD(0.05) A-0.018; B-0.028; C-0.025; A × B-NS; A × C-NS; B × C -0.056; A × B × C -0.079										

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 10. Effect of different organic sources on the heavy fraction organic carbon of sub-surface soil

Heavy fraction organic carbon of soil (g kg ⁻¹)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	10.20	9.90	13.90	34.10	38.10	9.90	9.80	13.80	34.00	38.00
N ₁	3.50	34.10	23.80	8.40	12.70	3.30	34.00	23.70	8.10	12.60
N ₂	27.30	29.00	13.40	27.60	7.10	26.00	28.80	13.30	27.50	7.00
N ₃	32.40	24.90	4.50	32.20	21.30	32.00	24.70	4.30	32.00	21.10
CD (0.05) A-0.011; B-0.017; C-0.015; A × B - NS; A × C- NS; B × C - 0.033; A × B × C - NS										

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 11. Effect of different organic sources on mineral-associated organic carbon of surface soil

Mineral-associated organic carbon (g kg ⁻¹)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	0.30	0.90	1.10	0.70	0.60	0.40	1.10	1.30	1.00	1.00
N ₁	0.30	1.20	0.50	0.50	1.20	0.30	1.40	0.70	0.70	1.50
N ₂	0.20	0.50	0.70	0.70	1.40	0.40	0.60	1.00	1.20	1.70
N ₃	0.40	1.00	0.80	0.80	0.40	0.60	1.20	1.20	1.10	0.80
CD(0.05)	A-0.004; B-0.006; C-NS;			A × B-0.008;		A × C-NS;		B × C -0.011;		A × B × C-NS

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Table 12. Effect of different organic sources on mineral-associated organic carbon of sub-surface soil

Mineral-associated organic carbon (g kg ⁻¹)										
Treatments	Virippu					Mundakan				
	NOM	FYM	ART	DNC	RHB	NOM	FYM	ART	DNC	RHB
N ₀	0.80	0.50	1.00	0.70	0.80	0.90	0.60	1.30	0.90	1.00
N ₁	0.70	1.10	1.00	0.50	0.70	0.80	1.40	1.20	0.60	0.90
N ₂	0.80	0.70	0.70	0.90	0.70	0.80	0.90	1.00	1.10	1.00
N ₃	0.90	0.80	0.80	1.30	0.70	1.00	1.10	0.90	1.40	0.80
CD(0.05)	A-0.004; B -0.007; C-0.006; A × B-NS; A × C - NS; B × C-0.014; A × B × C -NS									

A: cropping season; B: organic source; C: nitrogen level; NS: non significant; NOM: non organic manure; FYM: farmyard manure; ART: *Artocarpus heterophyllus* leaves; DNC: *Sesbania aculeata* leaves; RHB: rice husk biochar.

Discussion

Total organic carbon (TOC)

Surface soil total carbon content ranged from 1.72 to 4.06 %, while subsurface layers contained 1.11 to 3.98 %. Organic amendments, applied either independently or combined with NPK fertiliser, increased TOC at both 0–15 cm and 15–30 cm depths through external carbon inputs. During the virippu season, FYM application alone yielded the highest TOC value of 4.04 %, followed by RHB at 3.45 % (Table 3). Compared to NPK fertilisation, cattle manure and RHB generated substantially larger TOC increases in the 0–15 cm layer than *Artocarpus* or daincha amendments. Exogenous carbon from manure applications proved more effective for SOC sequestration; the surface soil TOC accumulation was primarily derived from organic residues, whereas for the subsoil, carbon originated from dissolved organic matter, root systems and root exudates (17). This is accelerated by chemical fertiliser application through increased rhizodeposition, root biomass and crop residue incorporation.

The experiment proceeded without soil acidity amelioration, as liming increases soil respiration and depletes SOC stocks (18). In acidic soils, lime reacts with active acidity (H⁺, Al³⁺, CO₂) in solution and reserve acidity on colloid exchange sites, releasing CO₂. Carbonate mineral dissolution functions as either a CO₂ source or sink (19). At pH < 5, H⁺ interaction with HCO₃⁻ generates CO₂. During NH₄⁺ nitrification to NO₃⁻, strong acids like HNO₃ promote carbonate mineral dissolution, producing CO₂: CaMg(CO₃)₂ + 4HNO₃ → Ca²⁺ + Mg²⁺ + 4NO₃⁻ + 2CO₂ + 2H₂O. This negative feedback mechanism in SOC turnover and carbon cycling depends on climatic factors, including carbon input rates and temperature.

Soil carbon fractions

Soil carbon fractions under different nutrient management regimes indicate that integrated nutrient management consistently produced higher carbon pool values than chemical fertilisers alone (Tables 5–12). The combined application of chemical fertiliser and

manure increased LFOC and POC fractions in TOC within paddy soils under alternate wetting-drying cycles (20). In cold and semiarid regions, labile particulate SOC constitutes approximately 50 % of total SOC and responds most strongly to management interventions (21). Conversely, hot, humid environments stimulate microbial activity, intensifying decomposition and humification of labile SOC fractions (22). Such intensive humification in humid tropics transforms labile carbon into chemically recalcitrant aromatic pools. In rice systems, prolonged submergence promotes passive SOC pool formation despite potentially limited microbial activity. These findings align with the observation in rice cultivation systems, 29 % of applied organic carbon stabilised into recalcitrant pools (2).

Rice husk biochar enhanced cPOC by 350 % over mineral N alone, compared to 150 % under ART. The RHB plus the highest N rate combination produced maximum cPOC enrichment (575 %). Fine POM exhibited lower carbon concentration (2.26–5.80 %) than coarse POM (1.6–23.6 %). However, fPOC pools were 2 to 4-fold smaller than cPOC under organic treatments (Table 7). Relative to mineral fertilisation, ART and RHB expanded fPOC pools by 50 and 500 % respectively. Both cPOC and fPOC diminished with depth. Subsurface amended plots contained lower cPOC than mineral-fertilised plots. Organic amendments also significantly reduced subsurface fPOC relative to mineral N ($p < 0.05$).

Nutrient treatments produced more pronounced differences in LFOC and POC fractions than in total SOC. Elevated POM levels indicate carbon and nutrient retention in intermediate-availability pools, preventing losses while maintaining accessibility. Rice husk biochar (RHB) application achieved this outcome. Surface soil LFOC exhibited the highest carbon concentration (36.20–171.30 g kg⁻¹), exceeding iLFOC, HFOC and MinOC. Organic amendments substantially elevated LFOC concentrations relative to inorganic fertilisers. The RHB treatment expanded the LFOC pool by 150 % and the iLFOC pool by 175 % compared to integrated treatments incorporating FYM, *Artocarpus* and daincha. While RHB did not significantly affect HFOC and MinOC, other organic sources

enhanced these fractions. Light fraction organic matter consists primarily of organic residues at various decomposition stages and microbial debris (23). Its constituents undergo rapid mineralisation due to their labile character and minimal protection from soil colloids (24). Daincha incorporation minimally influenced LFOC and iLFOC fractions. Plant residues and organic amendments increase total organic carbon and associated POM carbon (25). Low C/N ratio residues (N-rich materials) decompose rapidly, limiting POM accumulation. Soil disturbances, including aggregate disruption through cultivation and wet-dry cycling, expose organic matter to microbial decomposition, reducing POM content. Surface horizons contained greater organic carbon than subsurface layers. Elevated SOC concentrations in the A horizon increased the substantial carbon storage within particulate organic matter pools (3).

Conclusion

Organic amendment application in rice-rice systems substantially influences soil organic carbon (SOC) distribution across carbon pools. Physical soil organic matter (SOM) fractions exhibit differential management responses. Although coarse particulate organic carbon (cPOC) constitutes a minor SOC proportion, it demonstrates greater management sensitivity than fine particulate organic carbon (fPOC), indicating its function as a more labile carbon pool. Density-fractionated carbon pools responded significantly to season and amendment type, with mundakan values exceeding virippu for all fractions except the heavy fraction, where changes were minimal. They occur in the sequence LFOC > iLFOC > HFOC > MinOC. Surface horizons contained greater concentrations across all fractions, while unamended treatments increased subsurface mineral-associated organic carbon. Management sensitivity decreased in the order LFOC and iLFOC > sand-sized HFOC > and clay-sized MinOC, suggesting these fractions correspond to active, slow and passive SOC pools respectively. While organic sources benefited all pools, biochar combined with nitrogen at all rates produced the strongest effects on cPOC, LFOC and iLFOC carbon content, resulting in a short-term increase in soil fertility. The presence of higher amounts of fPOC, recalcitrant HFOC and MinOC in soil increase the carbon stocks and helps to combat the adverse effects of climate change.

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Authors' contributions

RK carried out the experiment and statistical analysis. BB contributed to the design of experiments and verification of results and the discussion part. SS suggested a procedure to fractionate soil carbon and provided the required facilities to carry out. All authors have read and approved the final manuscript.

Compliance with ethical standards

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