

Article

Decoupled Carbon and Nitrogen Cycling Across Soil Particle-Size Fractions in Apple Orchards of the Jiaodong Peninsula, China

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Abstract

The coupled mechanisms governing carbon–nitrogen turnover across soil particle-size fractions remain unclear. This study investigated soil organic carbon (SOC) and total nitrogen (TN) dynamics across five particle-size fractions in Cambisols under conventional and organic orchard management. Results showed that particle size dominated $\delta^{13}\text{C}$ variation (Partial $\eta^2 = 0.36$) while management practice regulated $\delta^{15}\text{N}$ variation (Partial $\eta^2 = 0.38$), revealing a fundamental decoupling of C and N cycling within the particle-size matrix. The progressive linear increase in $\delta^{13}\text{C}$ with declining particle size signaled a transition of SOC from net accumulation to net decomposition, accompanied by directional carbon translocation from coarse sand to clay fractions. A universal critical soil pH threshold ~ 5.3 was identified, where inter-particle-size carbon flow reached its maximum while microbial decomposition was minimized. Organic management reduced the intensity of plant-derived carbon translocation between particle-size fractions, yet substantially enhanced microbial anabolism, leading to drastically elevated stocks of microbial necromass carbon (MNC) in both particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) pools. Notably, the relative proportional distribution of POC and MAOC remained unchanged across the two management practices, which was intrinsically constrained by the inherent textural properties of the studied Cambisols. Counterintuitively, progressive soil acidification concurrently increased SOC lability and overall carbon stabilization, a paradox that directly demonstrated decoupling between chemical oxidizability and physical protection during particle-size carbon translocation. These findings confirmed incomplete carbon–nitrogen coupling within soil particle-size fractions, and demonstrated that SOC stabilization was co-regulated by organo-mineral interactions and microbial processing, whereas nitrogen dynamics were primarily modulated by exogenous management-derived inputs. This work provided novel insights for optimizing agricultural management strategies to synergistically enhance soil fertility and long-term carbon sequestration.



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Keywords: soil particle-size fractionation; stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$); C–N decoupling; carbon translocation; Cambisol; critical pH threshold

1. Introduction

Soil is the largest terrestrial carbon pool, storing a median of 1460.5 Pg of organic carbon (SOC) within the top 1 m layer [1]. Subtle shifts in this storage capacity (e.g., annual increase of 4‰) can profoundly regulate atmospheric CO₂ concentrations and mitigate climate change [2]. The capacity of soil as a carbon sink is fundamentally mediated by organic matter–mineral interactions within distinct particle-size fractions, yet the mechanisms governing carbon and nitrogen stabilization across these fractions remain poorly studied.

For decades, classical theory has posited a strict synchronous coupling between SOC and total nitrogen (TN). This framework resets on the premise that microbial metabolic processes maintain these two elements in stoichiometric balance during organic matter decomposition and transformation [3–6]. This paradigm has guided numerous agricultural management strategies, e.g., often targeting specific C:N ratios, which aimed at simultaneously optimizing nutrient cycling and carbon sequestration [7].

However, emerging evidence from natural abundance stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) challenges this universal coupling framework. For instance, a decade-long application of pig slurry and deep litter to Typic Hapludult in southern Brazil increased $\delta^{15}\text{N}$ values across all particle-size fractions, whereas $\delta^{13}\text{C}$ remained unchanged [8]. According to prevailing models, newly formed organic carbon is initially sequestered in larger particle-size fractions in the form of particulate organic carbon (POC). It subsequently undergoes microbial decomposition, and is transferred to silt–clay fractions as mineral-associated organic carbon (MAOC), where it receives physicochemical protection [9]. This conceptual flow of carbon from coarse to fine particle fractions align with the aggregate hierarchy theory of SOC stabilization [10–12]. Notably, Tou et al. [12] demonstrated that plant residue quality (e.g., phosphorus and cellulose content) served as a key regulator governing the stabilization efficiency of residue-derived carbon across different particle-size classes. Conversely, Liu et al. [13] reported that mineral fertilization could slow the rate of this inter-particle carbon translocation process.

In contrast, nitrogen cycling processes across different particle-size fractions are far more complex, as they involve not only microbial transformation but also inorganic nitrogen mobility and their potential preferential enrichment within specific fractions [14]. Consistent with this mechanistic difference, positive correlations have been observed between soil $\delta^{15}\text{N}$ and available inorganic nitrogen, as well as C:N ratios [15,16]. Marchetti et al. [17] further suggested that a C:N ratio below 9 may indicate prevailing oxidation processes and accelerated SOC decomposition, whereas a C:N ratio exceeding 12 could reflect insufficient nitrogen for efficient humification. Collectively, these findings imply that decoupling between the cycling dynamics of SOC and TN may occur under specific soil conditions.

Although the decoupling dynamics between SOC and TN have been reported in extreme environments such as arid ecosystems [18–20], this phenomenon remains scarcely documented in intensively managed agricultural soils, and critical knowledge gaps persist. First, the relative contributions of inherent soil physical protection versus management practices to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation across particle-size fractions remain unquantified, hindering a mechanistic understanding of whether carbon and nitrogen are governed by distinct regulatory filters. Second, it remains unclear whether carbon and nitrogen synchronously translocate across the entire particle-size gradient, or if their inter-flow pathways exhibit differential sensitivities to key environmental factors such as soil pH.

To address these gaps, we conducted a comparative analysis of natural abundance $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, alongside microbial necromass carbon (MNC), across a full, continuous particle-size gradient under conventional mineral fertilization and organic management regimes. This study aimed to test two core hypotheses: (1) Particle-size and management

practice exerted divergent controls on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation across fractions, with organo-mineral physical protection dominating carbon fractionation, while exogenous nutrient inputs regulated nitrogen isotope dynamics; and (2) carbon and nitrogen flow across the entire particle-size fractions exhibited distinct decoupling responses to soil pH, with carbon displaying a distinct critical threshold that was absent for nitrogen.

This work provided direct empirical evidence for carbon–nitrogen decoupling in agricultural soils, challenging the long-held assumption of their uniform co-regulation. Our findings offered practical implications for the sustainable management of intensive cropping systems, proposing a novel framework for optimizing soil fertility and climate change mitigation through the targeted regulation of carbon flow pathways without disrupting nitrogen cycling.

2. Materials and Methods

2.1. Study Area

This study was conducted in apple orchards located in Yantai City, on the northeastern Jiaodong Peninsula, China. The region is characterized by hilly and low mountain terrain and features a warm temperate, semi-humid monsoon climate, which has supported over a century of apple cultivation. The dominant soil type is brown earth, classified as Cambisol with a sandy loam texture [21], covering approximately 83% of the cultivated area. The parent material is predominantly granite bedrock, with hydromica and vermiculite as the primary minerals [22]. Soil acidification has been observed due to excessive fertilization, with a decrease of 1.5 pH units (from 6.8 to 5.34) in orchard soils and 0.4 pH units (from 6.8 to 6.39) in cropland soils over the past decade [23]. A significant positive correlation between SOC and soil pH suggests that ongoing acidification may accelerate SOC loss [24].

2.2. Soil Sampling and Analysis

A total of 16 soil sites (12 under conventional management and 4 under organic management) with varying pH levels were sampled from apple orchards in Yantai. At each site, three composite soil samples (replicates) were collected from five cores of topsoil (0–20 cm depth) using a stainless-steel auger (3 cm diameter). Samples were stored in polyethylene bags and transported to the laboratory for analysis. Geographic coordinates, elevation, and the duration of apple cultivation at each site were recorded (Table 1).

Table 1. Sampling site information and soil basic properties.

Management	No.	County	Coordinates		Elevation (m)	Slope (°)	Topography	pH (H ₂ O)	SOC (g/kg)	TN (g/kg)	Particle-Size Distribution (%) ¹			Tree Age (Year)
			Latitude	Longitude							Sand	Silt	Clay	
Conventional fertilization	1	Huangtulang	37°25'47'' N	121°1'23'' E	73	3.23	Flatland	3.78	9.70	1.54	73.15	19.89	6.95	10
	2	Hexikuang	37°15'28'' N	120°57'6'' E	215	0.33	Flatland	3.89	11.03	1.61	78.87	17.45	3.68	25
	3	Shanggaojia	37°21'48'' N	120°56'31'' E	240	8.33	Tableland	4.01	7.70	0.90	88.51	9.54	1.94	23
	4	Shangzhuangtou-1	37°15'47'' N	120°55'42'' E	170	2.82	Tableland	4.21	9.34	1.14	72.10	22.35	5.55	25
	5	Jianjishan	37°14'22'' N	120°52'56'' E	230	7.62	Flatland	4.44	11.64	1.68	90.81	7.20	2.00	30
	6	Shangyaogou	37°20'2'' N	120°58'48'' E	142	1.97	Terrace	4.58	13.75	2.17	72.52	22.93	4.55	22
	7	Ekuang	37°16'33'' N	121°0'9'' E	214	7.89	Terrace	4.76	11.97	1.31	83.11	14.18	2.71	18
	8	Dayuzhuang-1	37°17'49'' N	120°40'2'' E	142	0.73	Tableland	4.95	9.40	0.96	83.48	13.67	2.85	23
	9	Shangzhuangtou-2	37°15'40'' N	120°55'48'' E	275	10.08	Tableland	6.23	9.86	1.30	80.02	16.61	3.37	25
	10	Wuli-1	37°38'28'' N	120°57'54'' E	63	2.63	Tableland	6.57	11.71	1.66	71.37	25.62	3.01	8
	11	Gaoling	37°17'54'' N	121°31'9'' E	40	1.04	Flatland	6.82	7.56	0.68	85.22	11.87	2.91	3
	12	Xiaomajia	37°18'25'' N	120°58'26'' E	191	6.25	Terrace	6.84	12.11	1.49	79.17	17.88	2.95	15

Table 1. Cont.

Management	No.	County	Coordinates		Elevation (m)	Slope (°)	Topography	pH (H ₂ O)	SOC (g/kg)	TN (g/kg)	Particle-Size Distribution (%) ¹			Tree Age (Year)
			Latitude	Longitude							Sand	Silt	Clay	
Organic fertilization	13	Dayuzhuang-2 (Carpet)	37°18'0" N	120°39'57" E	140	0.83	Flatland	6.95	14.45	1.63	88.91	8.92	2.17	20
	14	Dayuzhuang-3 (Natural grass)	37°18'2" N	120°39'59" E	146	1.22	Tableland	7.04	21.97	2.48	58.78	32.67	8.56	28
	15	Dayuzhuang-4 (Vicia villosa)	37°18'5" N	120°39'57" E	138	0.68	Flatland	7.35	18.33	2.17	89.71	8.94	1.34	15
	16	Wuli-2 (Vulpia myuros)	37°38'34" N	120°58'14" E	65	3.67	Tableland	7.12	14.54	1.68	74.51	16.34	9.15	10

¹ Sand (20–2000 µm); silt (2–20 µm) and clay (<2 µm).

Upon arrival, samples were air-dried. One portion of each bulk sample was set aside for particle-size distribution analysis, while the remainder was ground and passed through a 2 mm sieve for bulk soil analysis. According to standard soil analysis procedures, SOC content in both bulk soil and particle-size fractions was determined using the K₂Cr₂O₇ oxidation method; TN was analyzed by the Kjeldahl method. Soil pH was measured at a soil-to-water ratio of 1:2.5 using a pH meter (PHSJ-4F, INESA, Shanghai, China). Basic soil properties were listed in Table 1.

2.3. Soil Particle-Size Fractionation

Soil particles were separated into five distinct size classes using a combination of wet sieving and sedimentation techniques: coarse sand (2000–250 µm), fine sand (250–53 µm), coarse silt (53–20 µm), fine silt (20–2 µm) and clay (<2 µm) [25,26]. For subsequent analysis, SOC and TN were distinguished within three aggregate classes: macro-aggregates (2000–250 µm), micro-aggregates (250–53 µm), and silt–clay fractions (<53 µm), or within two functional pools: particulate (>53 µm) and mineral-associated (<53 µm) fractions [6,27,28].

Wet sieving was performed using a 4-sieve tower (mesh sizes: 2000, 250, 53, and 20 µm) connected to a circulating water pump, with deionized water in a beaker (F-SW200, FOCUCY, Changsha, Hunan, China). For particles smaller than 20 µm, size distribution was determined by sedimentation in a 1000 mL cylinder following Stokes' law.

For fractionation and subsequent analysis, 100 g of air-dried, unground soil was used per replicate. The procedure was repeated three times to ensure accuracy. Separated fractions were analyzed for their content of SOC, TN, stable isotopes (δ¹³C and δ¹⁵N), and oxidizable organic carbon.

2.4. Stable Isotope Analysis

Prior to analysis, all bulk soil and particle-size fraction samples were acidified with 2M HCl for 24 h to remove carbonates [29]. Isotope ratios (¹³C/¹²C and ¹⁵N/¹⁴N) were determined using a stable isotope ratio mass spectrometer (MAT253, Thermo Fisher, Waltham, MA, USA). Vienna Pee Dee Belemnite (VPDB) and atmospheric N₂ (AIR) were used as the standards for carbon and nitrogen, respectively. The δ¹³C and δ¹⁵N values (‰) were calculated using the following equation:

$$\delta = \frac{R_{\text{SAMPLE}} - R_{\text{STD}}}{R_{\text{STD}}} \times 1000 \quad (1)$$

where R_{SAMPLE} and R_{STD} are the isotope ratios of the sample and the standard, respectively. Each sample was analyzed in triplicate, with a measurement precision of 0.1‰ for δ¹³C and 0.2‰ for δ¹⁵N [30].

To assess the dynamics of carbon and nitrogen across particle-size fractions, the difference in δ¹³C (or δ¹⁵N) between a specific particle-size fraction and the bulk soil was

calculated and denoted as $\Delta\delta^{13}\text{C}$ (or $\Delta\delta^{15}\text{N}$). A negative $\Delta\delta$ value reflects the accumulation/enrichment of the heavier isotope (^{13}C or ^{15}N) in that fraction, while a positive $\Delta\delta$ value suggests the decomposition/loss processes. Similarly, the differences in $\delta^{13}\text{C}$ (or $\delta^{15}\text{N}$) among different particle-size fractions were used to infer the direction and intensity of SOC and TN flow between fractions, with a smaller inter-fraction difference indicating a higher probability of transfer [13,31].

2.5. SOC Lability in Particle-Size Fractions

SOC within each particle-size fraction was chemically fractionated into four oxidizable pools using a modified Walkley–Black method: very labile carbon (VLC), labile carbon (LC), less labile carbon (LLC), and non-labile carbon (NLC) [32]. Briefly, 0.5 g of soil from each size fraction was mixed with 10 mL of 0.167 M $\text{K}_2\text{Cr}_2\text{O}_7$ and varying volumes (5, 10, and 20 mL) of concentrated H_2SO_4 (18 M) in a 500 mL conical flask. The mixture was kept in the dark for each oxidation step.

To evaluate the decomposability and relative stability of SOC across the different particle-size fractions, the SOC lability (L) and lability index (LI) were calculated using the following equations [33,34]:

$$L = \frac{\text{VLC}}{\text{total SOC} - \text{VLC}} \quad (2)$$

$$\text{LI} = \frac{\text{VLC} \times 3 + \text{LC} \times 2 + \text{LLC} \times 1}{\text{total SOC}} \quad (3)$$

2.6. SOC Stabilization Pathways

To distinguish the relative contributions to different pathways to long-term SOC stabilization, SOC was partitioned into plant-derived and microbial-derived components [35,36]. MNC, representing the dominant microbial-derived SOC pool, was quantified via amino sugar (AS) analysis [34,37]. In brief, soil was hydrolyzed with 6 M HCl at 105 °C for 8 h, after which the solution was filtered, adjusted to neutral pH, and centrifuged. The supernatant was freeze-dried, and AS was extracted using methanol and subsequently derivatized into aldononitrile acetate derivatives. The derivatized extracts were then dried under an N_2 condition, and re-dissolved in a hexane-ethyl acetate mixture ($v/v = 1:1$) for quantification by gas chromatograph (7890B, Agilent, Santa Clara, CA, USA).

Fungal necromass carbon (FNC) and bacterial necromass carbon (BNC) were calculated from the measured concentrations of glucosamine (Glu) and muramic acid (Mur), respectively. The total MNC was obtained as the sum of FNC and BNC, using the following equations:

$$\text{FNC}(\text{g/kg}) = \left[\frac{\text{Glu}(\text{mg/g})}{179.17} - 2 \times \frac{\text{Mur}(\text{mg/g})}{251.23} \right] \times 179.17 \times \frac{9}{1000} \quad (4)$$

$$\text{BNC}(\text{g/kg}) = \text{Mur}(\text{mg/kg}) \times \frac{45}{1000} \quad (5)$$

$$\text{MNC}(\text{g/kg}) = \text{FNC} + \text{BNC} \quad (6)$$

where 179.17 and 251.23 are the molecular weights of glucosamine and muramic acid, respectively, and the coefficients 9 and 45 are the conversion factors from amino sugar to necromass carbon for fungi and bacteria, respectively.

2.7. Statistical Analysis

Two-way analysis of variance (ANOVA) was performed in IBM SPSS 25.0 (SPSS Inc., Chicago, IL, USA) to test the interactive effects of management practice and particle-size class on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. An F-protected least significant difference (LSD) post hoc test was applied for pairwise comparisons of treatment means, with significance defined

at $p < 0.05$ and $p < 0.01$. Curve fitting and all graphical outputs were generated using SigmaPlot 14.0 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Soil Particle-Size Fractions

The two-way ANOVA revealed significant interactive effects of particle size and management practice on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values associated with soil particle-size fractions ($p < 0.01$). However, their relative contributions diverged distinctly: particle size imposed a stronger control on $\delta^{13}\text{C}$ variation (Partial $\eta^2 = 0.36$), while management practice dominated the variation in $\delta^{15}\text{N}$ (Partial $\eta^2 = 0.38$) (Table 2).

Table 2. Two-way ANOVA test for the effects of soil particle-size and management practice on soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Source	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
	F	Sig	Partial η^2	F	Sig	Partial η^2
Corrected model	20.63	0.000	0.447	17.94	0.000	0.412
Intercept	67,324.17	0.000	0.997	692.61	0.000	0.751
Management practice	53.85	0.000	0.190	140.16	0.000	0.379
Particle-size	32.79	0.000	0.363	4.971	0.001	0.080
Practice $\times$ Size	6.546	0.000	0.102	2.148	0.076	0.036

The $\delta^{13}\text{C}$ values increased progressively with decreasing particle size (Figure 1a), and the linear shift in $\Delta\delta^{13}\text{C}$ from negative to positive values across the particle-size gradient (Figure 1b) indicated a functional transition of organic carbon from net accumulation in coarser fractions to net decomposition in finer fractions. Compared with conventional management (slope = 0.39), organic management facilitated greater carbon sequestration in coarse and fine sand fractions but accelerated carbon decomposition in the silt–clay fractions (slope = 1.04). This contrasting dynamic ultimately eliminated the significant management effect on $\delta^{13}\text{C}$ within the silt–clay fraction. In contrast, despite substantially higher $\delta^{15}\text{N}$ values under organic management, particle size showed negligible explanatory power for $\delta^{15}\text{N}$ variation (Figure 1c). Furthermore, the universally negative $\Delta\delta^{15}\text{N}$ values across all particle-size fractions indicated consistent nitrogen enrichment, with greater inter-particle-size variability observed under organic management (Figure 1d).

Concurrent with the carbon turnover transition from accumulation to decomposition, directional carbon flow (i.e., carbon transfer) from coarse sand to clay fractions was detected (Figure 2a,b). Conventional management exhibited more intense carbon translocation, particularly from fine sand to coarse silt ($\Delta\delta^{13}\text{C} = 0.06$) and from fine silt to clay ($\Delta\delta^{13}\text{C} = 0.14$) (Figure 2a). By comparison, organic management did not show enhanced inter-fraction carbon flow but instead sustained a higher degree of organic carbon decomposition across the size continuum (Figure 2b). Analogously, nitrogen flow from coarse sand to clay (and even reverse translocation) was observed, and this pattern was more pronounced under conventional management (Figure 2c,d).

Notably, carbon flow exhibited a well-defined critical pH threshold at approximately pH 5.3, where the magnitude of carbon translocation reached its maximum (Figure 3a–d). In contrast, no consistent critical pH threshold was identified for nitrogen flow across the particle-size fractions (Figure 3e–h), providing direct evidence that SOC and TN did not undergo synchronous-coupled changes during their accumulation, decomposition, and translocation across different particle-size fractions.

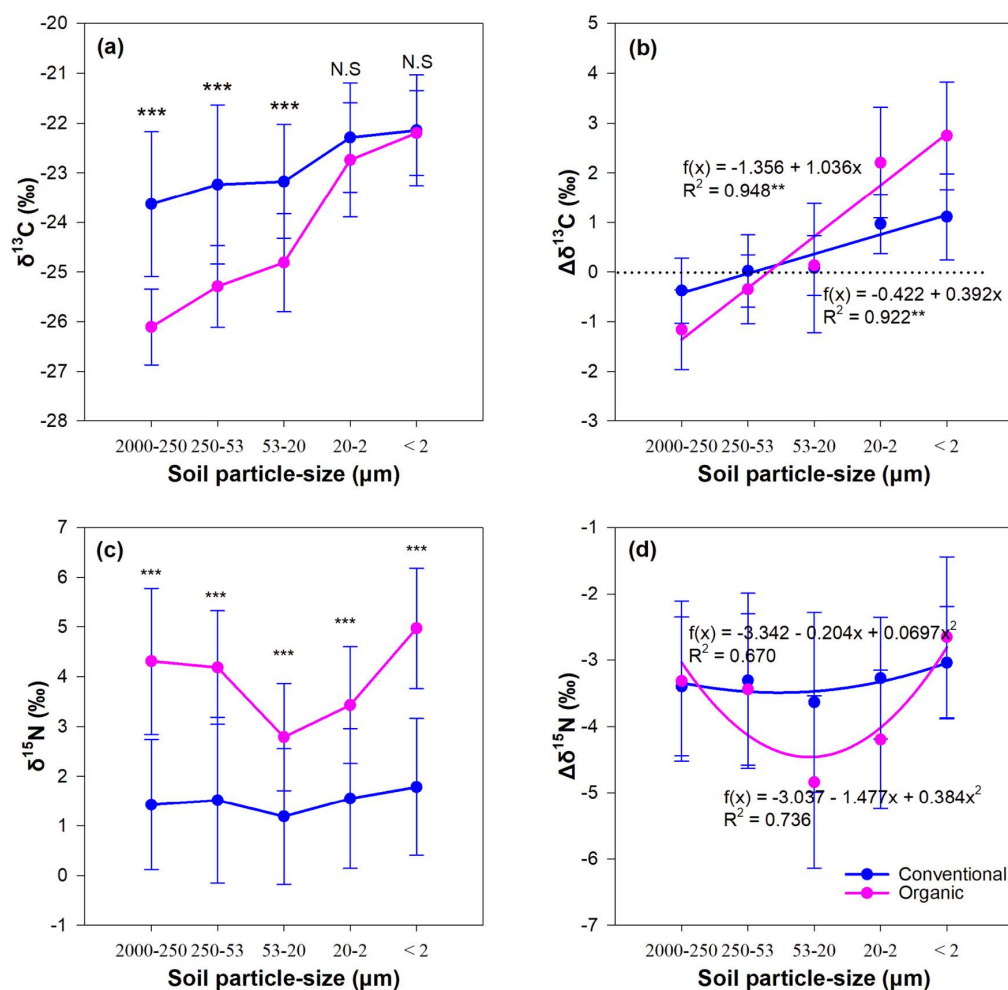


Figure 1. Variations in the composition of soil carbon and nitrogen stable isotopes along the gradient of decreasing particle-size fractions ((a): $\delta^{13}\text{C}$; (b): $\Delta\delta^{13}\text{C}$; (c): $\delta^{15}\text{N}$; (d): $\Delta\delta^{15}\text{N}$). Asterisks **, and *** denote significance levels at $p < 0.01$, and $p < 0.001$, respectively; N.S. indicates no significant difference.

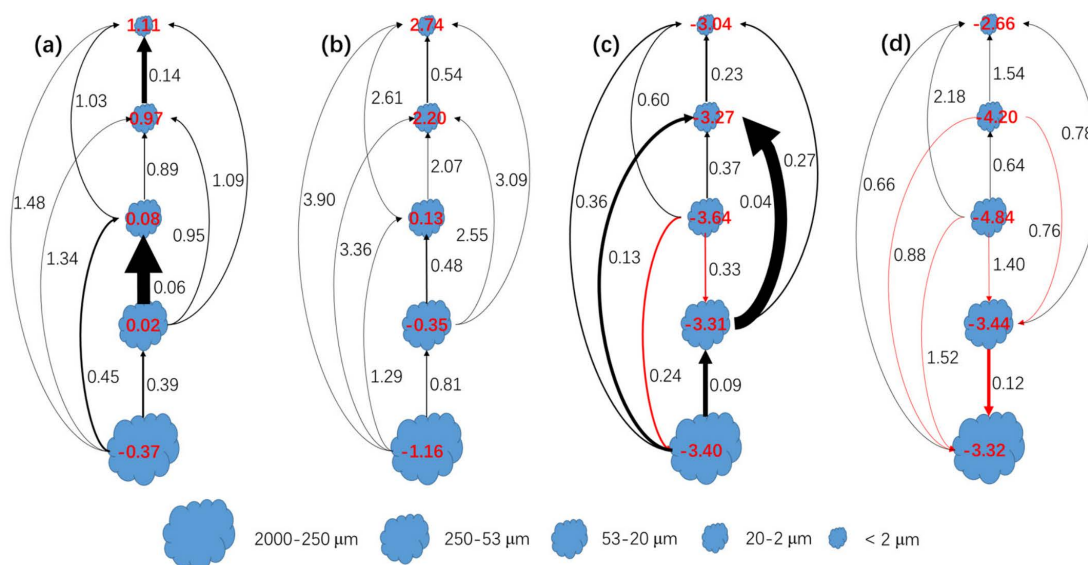


Figure 2. Probabilistic flow of carbon and nitrogen among soil particle-size fractions under conventional and organic management practices ((a): carbon flow under conventional; (b): carbon flow

under organic; (c): nitrogen flow under conventional, (d): nitrogen flow under organic). Arrow thickness indicates flow probability; black lines denote the flows from coarser to finer fractions, and red lines denote reversed flow paths.

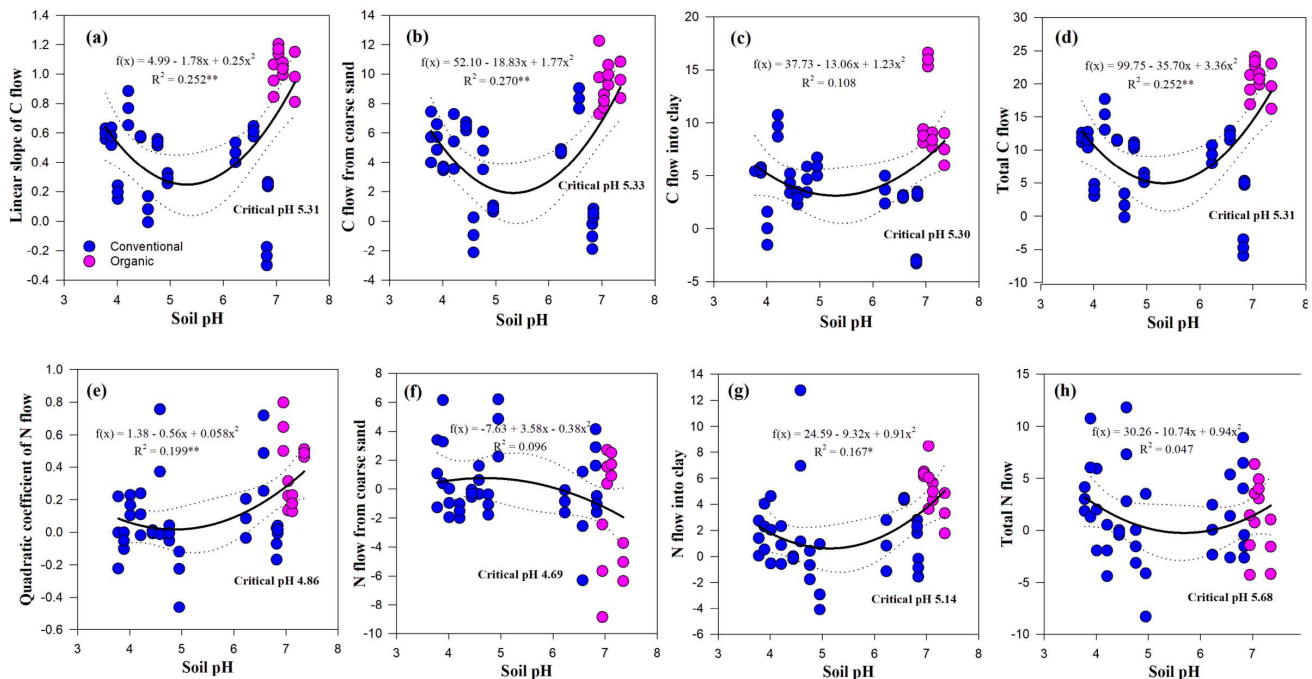


Figure 3. pH-dependent dynamics of carbon and nitrogen flows between soil particle-size fractions ((a): slope of carbon flow; (b): carbon flow from coarse sand; (c): carbon flow into clay; (d): total carbon flow; (e): coefficient of nitrogen flow; (f): nitrogen flow from coarse sand; (g): nitrogen flow into clay; (h): total nitrogen flow). Asterisks *, ** denote significance levels at $p < 0.05$ and $p < 0.01$, respectively.

3.2. Distribution of SOC and TN in Soil Particle-Size Fractions

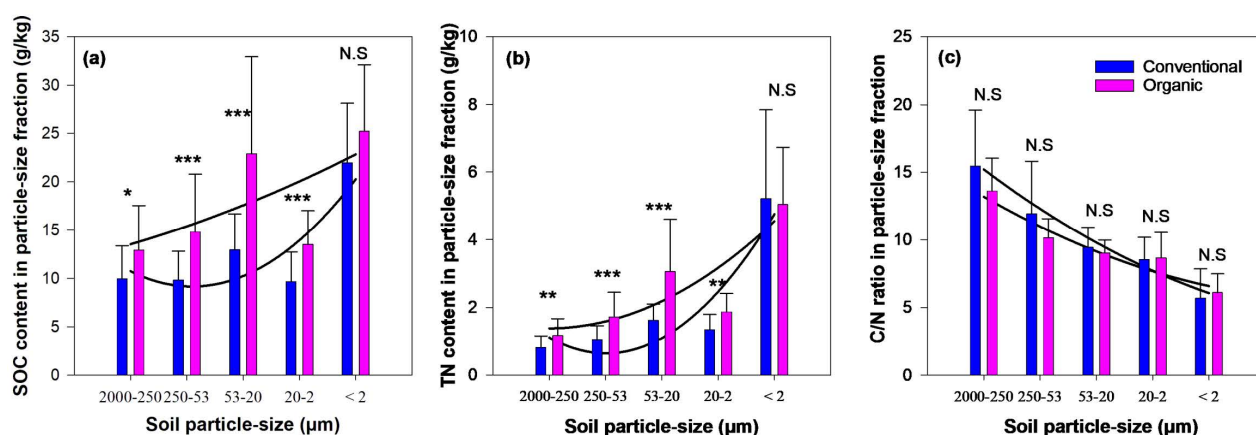
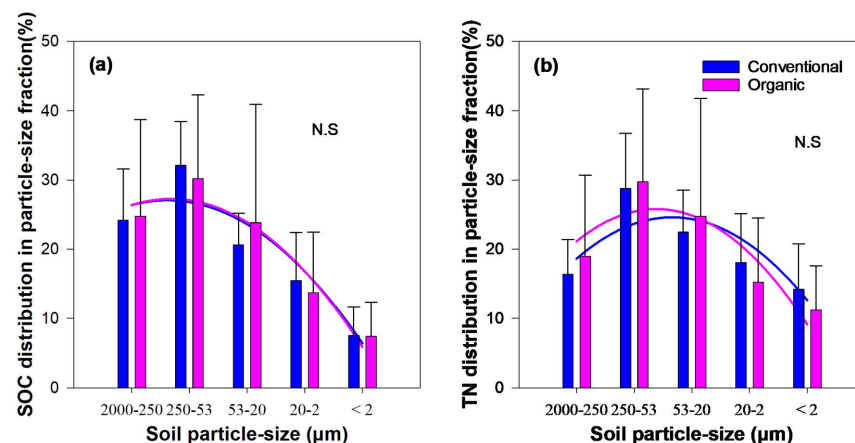
As summarized in Table 3, the overall recovery rates of SOC and TN, calculated as the sum of their concentrations in the five particle-size fractions relative to their bulk soil concentrations, ranged from 85.4% to 117.8% for SOC and 82.3% to 116% for TN. These recovery rates validated that the reliability of the particle-size fractionation protocol for characterizing SOC and TN dynamics. The concentrations of both particle-associated SOC and TN increased from the coarse sand to the clay fraction, with a more pronounced increase for TN (Figure 4a,b). Consequently, the C/N ratio exhibited a linear decline with decreasing particle size (Figure 4c). Moreover, particle-associated SOC and TN concentrations differed significantly between the two management practices across all fractions except the clay fraction, with consistently higher values under organic management (Figure 4a,b). However, their relative distribution proportions did not differ significantly between the two practices, and the clay fraction exhibited lower nutrient distribution due to the inherently lower clay content in the studied Cambisols (Figure 5).

Additionally, higher absolute stocks of particulate-associated SOC and TN (POC and PTN), and mineral-associated SOC and TN (MAOC and MATN) were detected under organic management, while their relative distribution proportions remained statistically comparable: 56.3% vs. 55.0% for POC, 43.7% vs. 45.0% for MAOC, 45.2% vs. 48.7% for PTN, and 54.8% vs. 51.3% for MATN, respectively (Figure 6).

Table 3. Concentrations of total organic carbon and total nitrogen in bulk soil and particle-size fractions.

No.	SOC			TN		
	Sum (g/kg)	Bulk (g/kg)	Recovery (%) ¹	Sum (g/kg)	Bulk (g/kg)	Recovery (%) ¹
1	8.65 ± 0.28 ²	9.70 ± 0.58	−10.82	1.27 ± 0.16	1.54 ± 0.10	−17.69
2	13.00 ± 0.96	11.03 ± 0.25	17.82	1.55 ± 0.20	1.61 ± 0.48	−3.77
3	6.98 ± 0.55	7.70 ± 0.27	−9.38	0.89 ± 0.10	0.90 ± 0.09	−1.16
4	10.72 ± 0.86	9.34 ± 0.32	14.80	1.10 ± 0.13	1.14 ± 0.02	−3.30
5	13.63 ± 0.35	11.64 ± 0.91	17.08	1.54 ± 0.09	1.68 ± 0.01	−8.35
6	13.29 ± 0.87	13.75 ± 1.66	−3.31	1.87 ± 0.17	2.17 ± 0.54	−13.79
7	13.86 ± 0.35	11.97 ± 0.91	15.81	1.35 ± 0.10	1.31 ± 0.05	2.97
8	8.03 ± 0.18	9.40 ± 0.13	−14.63	0.81 ± 0.03	0.96 ± 0.06	−15.94
9	9.84 ± 0.33	9.86 ± 0.40	−0.21	1.17 ± 0.05	1.30 ± 0.06	−9.99
10	11.57 ± 0.56	11.71 ± 0.97	−1.25	1.49 ± 0.08	1.66 ± 0.52	−10.16
11	7.85 ± 0.06	7.56 ± 0.57	3.84	0.70 ± 0.03	0.68 ± 0.02	1.94
12	11.24 ± 1.28	12.11 ± 1.92	−7.13	1.40 ± 0.29	1.49 ± 0.07	−5.47
13	15.18 ± 0.29	14.45 ± 2.67	5.03	1.50 ± 0.09	1.63 ± 0.53	−7.61
14	19.63 ± 0.90	21.97 ± 3.11	−10.67	2.54 ± 0.17	2.48 ± 0.28	2.72
15	16.00 ± 1.04	18.33 ± 2.20	−12.70	1.89 ± 0.07	2.17 ± 0.48	−12.67
16	13.63 ± 0.60	14.54 ± 3.32	−6.30	1.95 ± 0.02	1.68 ± 0.48	16.00

¹ Recovery = (sum−bulk)/bulk×100. ² Mean ± standard deviation (SD).

**Figure 4.** Distribution patterns of SOC content (a), TN content (b) and soil C:N ratio (c) along the gradient of soil particle-size fractions. Asterisks *, **, and *** denote significance levels at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; N.S indicates no significant difference.**Figure 5.** Proportional distribution of SOC (a) and TN (b) across different soil particle-size fractions. N.S indicates no significant difference.

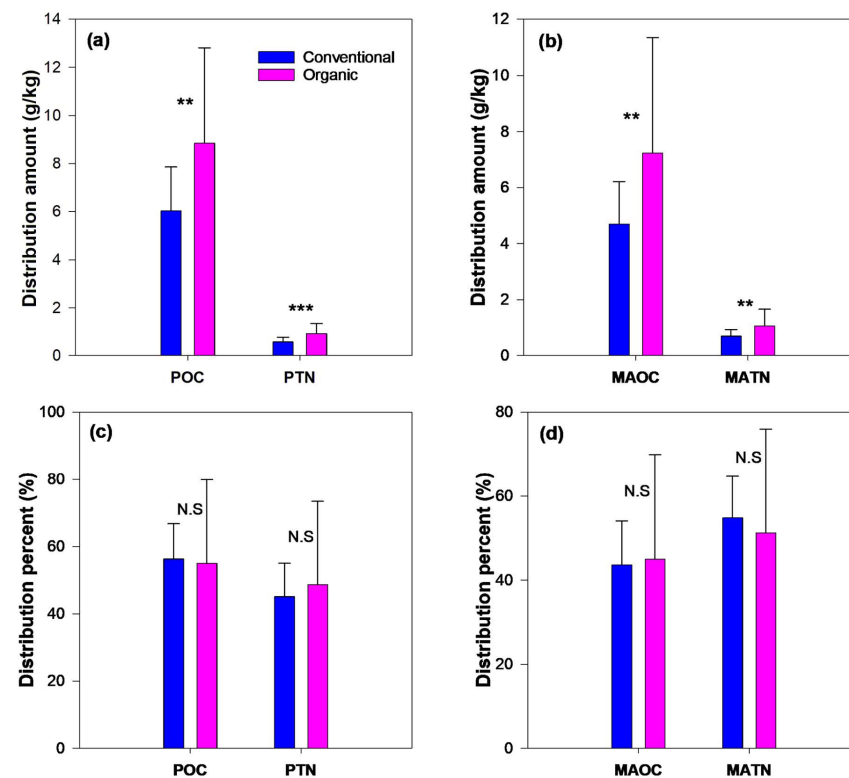


Figure 6. Absolute content and proportional distribution of particulate- and mineral-associated organic carbon and total nitrogen (POC, PTN, MAOC, MATN) across soil particle-size fractions ((a): POC and PTN content; (b): MAOC and MATN content; (c): distribution ratio of POC and PTN; (d): distribution ratio of MAOC and MATN). Asterisks **, and *** denote significance levels at $p < 0.01$, and $p < 0.001$, respectively; N.S. indicates no significant difference.

3.3. SOC Stability and Lability

Organic management significantly promoted the absolute content of MNC, including FNC and BNC (Figure 7a–c); nevertheless, the proportion of MNC relative to total SOC did not show significant differences between the two management regimes (Figure 7d–f). Furthermore, the relative contribution of microbial-derived carbon to SOC shared the identical critical pH threshold (approximately 5.3) with carbon flow (Figure 3a–d), demonstrating a tight coupling between organic carbon stability and its inter-particle translocation.

Given that MNC accounted for 29% of POC and 36% of MAOC in the 0–20 cm soil layer [38], we estimated a theoretical MNC:SOC ratio of 0.31, which was highly consistent with our measured value of 0.39. These validated coefficients were then applied to quantify the respective contributions of microbial-derived and plant-derived carbon to POC and MAOC pools (Figure 8). Under organic management, the absolute stocks of both microbial-derived and plant-derived carbon in POC and MAOC pools increased markedly, with microbial-derived carbon showing the most dramatic enhancement ($p < 0.001$) (Figure 8a,b). However, the relative proportion of plant-derived carbon decreased significantly, from 62.6% to 46.3% in POC and from 52.5% to 35.2% in MAOC, respectively (Figure 8c,d). This result further corroborated the observations in Figure 2, where reduced inter-particle-size carbon flow (dominated by plant-derived carbon) but enhanced decomposition (accompanied by greater microbial carbon anabolism) occurred under organic management. Additionally, the contributions of microbial-derived and plant-derived carbon to POC, MAOC and bulk SOC exhibited the same critical pH threshold (approximately 5.3) (Figure 9) as observed for MNC/SOC ratio (Figure 7d–f) and for carbon flow (Figure 3a–d). This strongly suggested that maximum organic carbon translocation and minimum microbial decomposition occurred concurrently at pH 5.3.

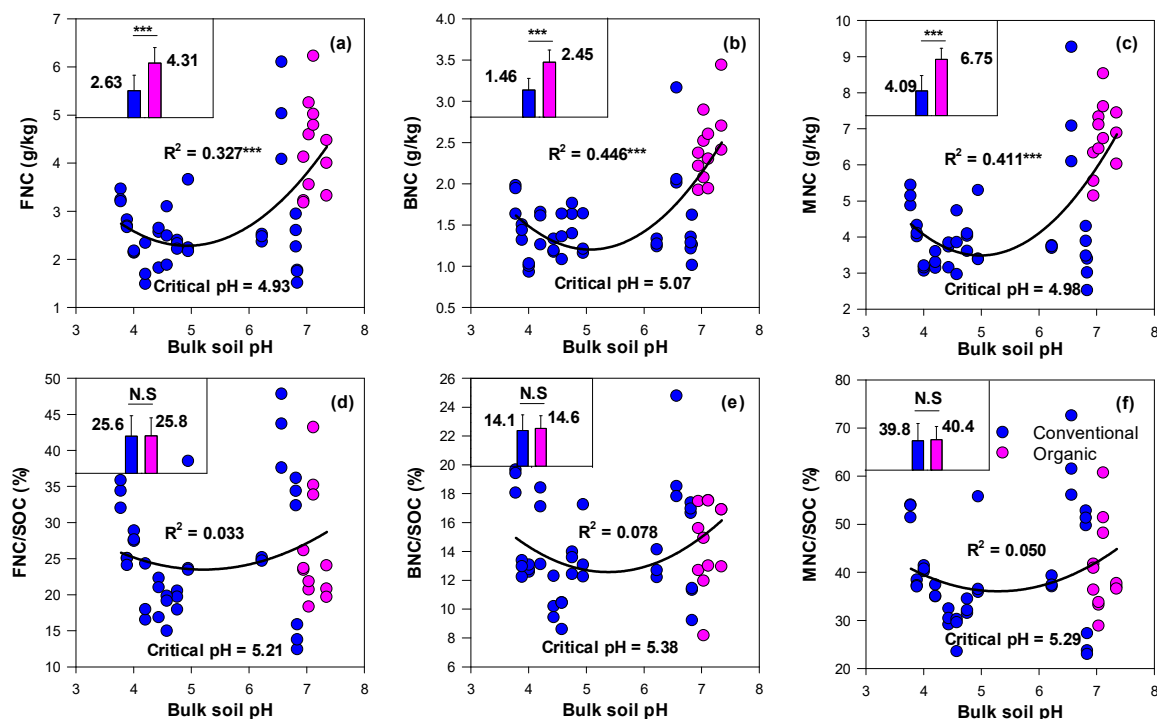


Figure 7. Changes in the absolute content and relative proportional contribution of microbial necromass carbon along a soil pH gradient ((a): FNC content; (b): BNC content; (c): MNC content; (d): FNC proportion; (e): BNC proportion; (f): MNC proportion). *** denotes significance level at $p < 0.001$, and N.S indicates no significant difference.

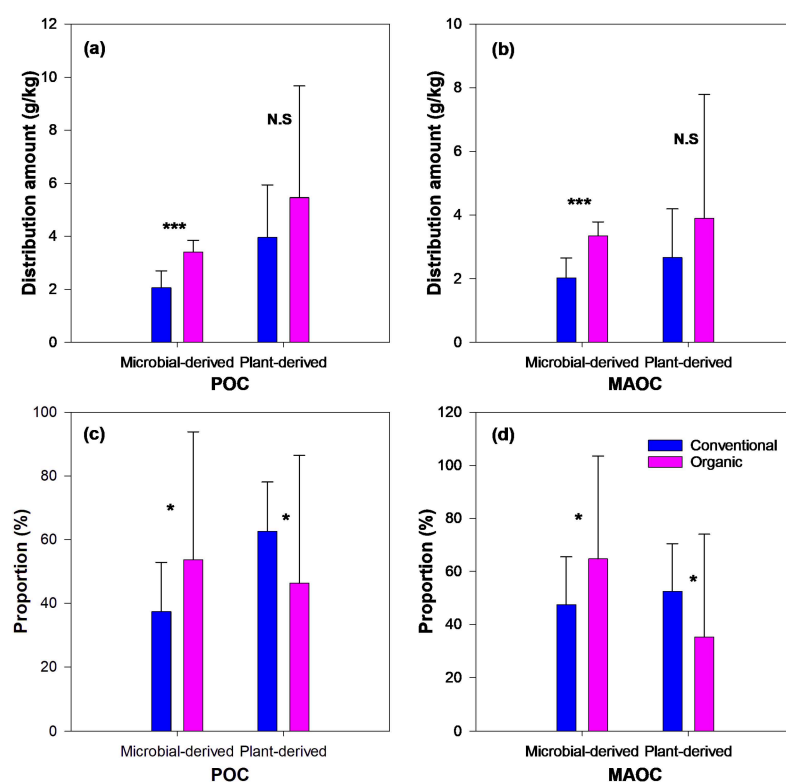


Figure 8. Absolute content and relative proportion distribution of microbial-derived and plant-derived carbon in particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) ((a): Carbon content in POC; (b): Carbon content in MAOC; (c): Carbon proportion in POC; (d): Carbon proportion in MAOC). Asterisks *, and *** denote significance levels at $p < 0.05$, and $p < 0.001$, respectively; N.S indicates no significant difference.

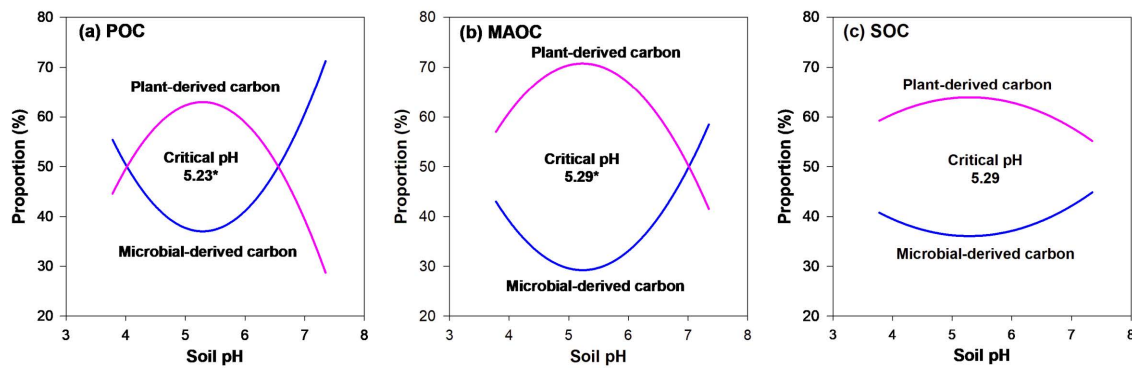


Figure 9. Shifts in the relative contributions of plant-derived (red line) versus microbial-derived (blue line) carbon to SOC fractions under soil acidification ((a): Particulate organic carbon (POC); (b): Mineral-associated organic carbon (MAOC); (c): Total SOC). * denotes significance level at $p < 0.05$.

Although no significant difference in particle-associated carbon lability (e.g., VLC, L and LI) was detected between the two management practices, contrasting linear trends were observed across particle-size fractions from coarse sand to clay: a continuous increase under conventional management versus a progressive decrease under organic management (Figure 10). This pattern suggested that carbon lability in the silt–clay fraction would be elevated under conventional management due to the intensified carbon translocation. Furthermore, the positive slope values describing the relationship between carbon lability and particle size increased significantly with progressive soil acidification under conventional management (Figure 11), indicating that the widespread acidification of the studied Cambisols would promote overall SOC lability.

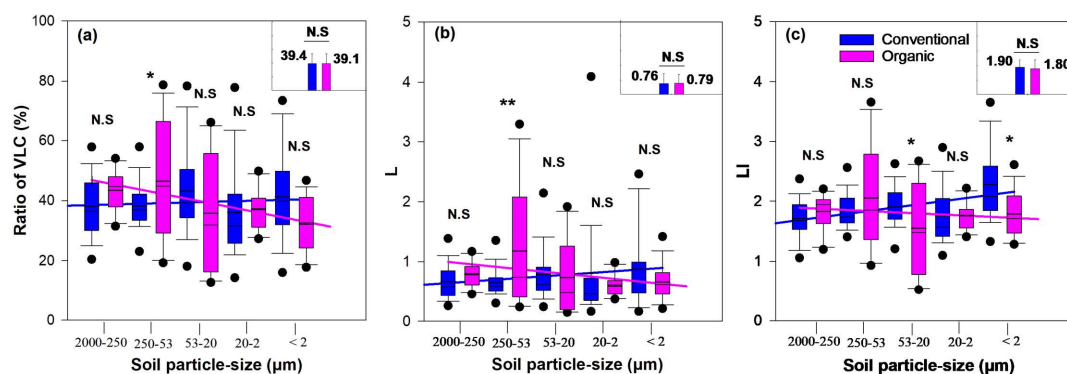


Figure 10. Changes in SOC lability characteristics along the soil particle-size gradient ((a): Very labile carbon (VLC); (b): Lability (L); (c): Lability index (LI)). * and ** denote significance levels at $p < 0.05$, $p < 0.01$, respectively; N.S indicates no significant difference.

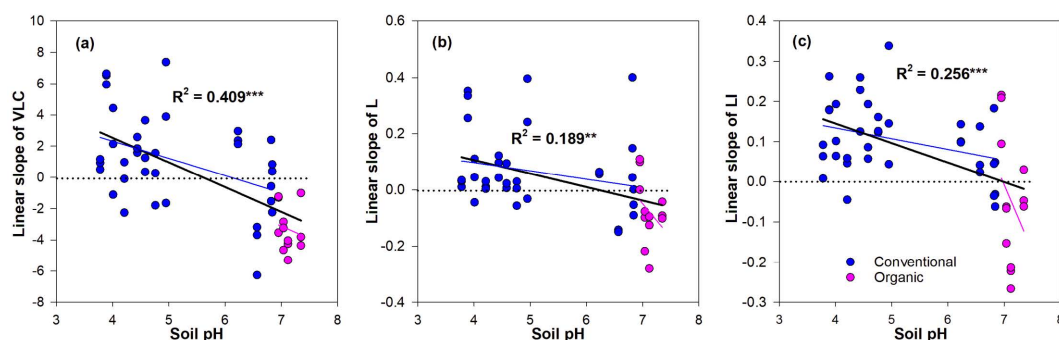


Figure 11. Linear relationships between soil acidification and the change rate of SOC lability characteristics across different soil particle-size fractions ((a): Very labile carbon (VLC); (b): Lability (L); (c): Lability index (LI)). ** and *** denote significance levels at $p < 0.01$ and $p < 0.001$, respectively.

4. Discussion

4.1. Divergent Controls on Stable Isotope Fractionation Reveal C-N Decoupling in Particle-Size Fractions

The distinct explanatory power of particle size (Partial $\eta^2 = 0.36$ for $\delta^{13}\text{C}$) versus management practice (Partial $\eta^2 = 0.38$ for $\delta^{15}\text{N}$) (Table 2) provided direct evidence that carbon and nitrogen cycles, as the two most critical fertility indicators, did not operate in strict synchrony across the particle-size hierarchy. This finding challenged the long-assumed ubiquitous coupling of the two elements in agricultural soils [3–5,39–41]. The progressive enrichment of $\delta^{13}\text{C}$ with decreasing particle size (Figure 1a,b) aligned with established conceptual models. Continuous microbial decomposition of organic substrates during physical occlusion within finer silt and clay fractions drove the preferential mineralization of ^{12}C , leaving residual organic carbon progressively enriched in ^{13}C . This isotopic gradient confirmed that the physical protection afforded by soil particles acted as the primary filter regulating carbon turnover, facilitating the progressive translocation of fresh plant-derived carbon from sand fractions toward more highly stabilized mineral-associated carbon in silt–clay fractions, which was consistent with the enrichment pathways of SOC within particle-size fractions reported in previous studies [13,42,43].

In contrast, the near-negligible influence of particle size but the highly significant effect of management practice on $\delta^{15}\text{N}$ (Figure 1c,d) demonstrated that nitrogen transformation pathways were largely decoupled from the physical structure of soil particles and instead depended on exogenous nitrogen inputs. This discrepancy could be attributed to the high mobility of inorganic nitrogen and its sensitivity to exogenous input regimes: organic management introduced nitrogen with higher $\delta^{15}\text{N}$ values via manure and crop-residue amendments, whereas chemical fertilizer application under conventional management rapidly homogenized lower and more uniform isotopic signals across all particle-size fractions. The consistently negative $\Delta\delta^{15}\text{N}$ values across all size classes (Figure 1d) further indicated that nitrogen enrichment occurred independently of the carbon accumulation–decomposition process, with organic management amplifying inter-particle-size variability by introducing heterogeneous organic nitrogen substrates that did not fully equilibrate across the particle-size continuum. Previous studies have also shown that $\delta^{15}\text{N}$ in agricultural soils was predominantly influenced by depleted nitrogen sources and losses (e.g., extractable inorganic N), with higher values under manure than under chemical fertilizer applications, as well as under higher available N conditions [8,15,44]. This discrepancy may arise because earlier classic models were developed for N-limited forest soils, where nitrogen cycling was tightly coupled to plant-derived carbon inputs, whereas in intensively managed agricultural soils, excessive exogenous nitrogen inputs broke this inherent synchrony.

Overall, our results demonstrated that district physical protection among particle-size fractions drove organic carbon isotope fractionation, governing its accumulation, decomposition and translocation; whereas the nutrient type and concentration of exogenous inputs dominantly regulated total nitrogen isotope fractionation, governing its transport and enrichment. This led to C-N decoupling within particle-size fractions.

The asymmetric translocation patterns further reinforced this non-synchrony: although conventional management exhibited more intense directional flow of both elements from coarse sand to clay fractions, it showed far greater divergence in the magnitude of nitrogen versus carbon transfer (Figure 2). Notably, a definitive critical pH threshold at approximately 5.3 was identified for maximum carbon flow (Figure 3) and minimum microbial transformation (Figures 7 and 9), while no parallel threshold emerged for nitrogen dynamics. Shi et al. [45] and Zhang et al. [46] both suggested a soil pH threshold around 5.5, which was very close to our observed value. Only Shi et al. [45] found that microbial

OTUs reached maximum abundance at pH 5.5, whereas Zhang et al. [46] reported that soil texture became the dominant factor regulating soil C-N stability and decomposition when pH exceeded 5.5. Mi et al. [47] also showed that soil pH significantly affected the distribution of ^{13}C in microbe communities. This pH-specific peak in carbon translocation likely corresponded to the trade-off in SOC dynamics between microbial decomposition, humification, and chemical sorption, where microbial community structure (including the fungal-to-bacterial ratio) and mineral surface reaction sites reached their optimal activation. In contrast, the absence of a consistent pH-regulatory effect on nitrogen reflected the complex interplay of ammonification, nitrification, and abiotic ammonium fixation, which lacked a unified pH optimum.

4.2. Asymmetric Accumulation of SOC and TN Across Particle-Size Fractions Further Supports C-N Decoupling

The more pronounced enrichment of TN relative to SOC along the decreasing particle size gradient resulted in a linear decline in the C/N ratio (Figure 4). This indicated that nitrogen underwent preferential sequestration in fine fractions. Possinger et al. [14] demonstrated that N enrichment occurred at both organo-mineral and organo-organic interfaces. Additional studies have also reported preferential stabilization of N at organo-mineral interfaces, which drove a substantial reduction in the C:N ratio [48,49]. This phenomenon arose because microbial nitrogen assimilation and mineral surface adsorption of ammonium proceed at faster rates than the stabilization of carbon, which required organic substrates to undergo partial decomposition before being incorporated into mineral-associated pools. This inherent temporal difference directly caused the asynchronous accumulation of carbon and nitrogen within particle-size fractions.

Notably, despite the substantially higher absolute concentrations of SOC and TN under organic management (Figure 4), the lack of significant differences in their relative distribution proportions across management practices (Figure 5) suggested that soil texture (particle-size hierarchy) imposed a fundamental structural constraint on nutrient partitioning. Even with elevated exogenous inputs, the inherently low clay content of the studied Cambisols limited the maximum nutrient storage capacities in silt-clay fractions, preventing further divergence in the relative distribution of carbon and nitrogen. This finding aligned with Lu et al. [50], who identified a clear hierarchical control: nutrient contents were primarily determined by silt and clay content. The parallel increases in absolute stocks of particulate-associated and mineral-associated carbon and nitrogen under organic management, with no shifts in their proportional contributions (Figure 6), further demonstrated that long-term organic inputs replenished all functional carbon and nitrogen pools simultaneously rather than selectively targeting specific fractions. However, the asynchronous shifts in their C-N distribution proportions further confirmed that nitrogen preferentially migrated and was lost from particulate fractions, and subsequently accumulated in more stable mineral fractions, with its turnover pathway being completely independent of carbon dynamics.

4.3. Reconciling the Paradox Between Enhanced Carbon Lability and Promoted Carbon Stabilization Under Progressive Acidification

The observed co-occurrence of enhanced particle-associated carbon lability (Figures 10 and 11) alongside diminished inter-particle carbon flow (Figure 3) and a relative decline in plant-derived carbon (Figure 9) under progressive soil acidification presented an apparent paradox. The increased carbon lability under acidification reflected a shift in the in situ chemical stability of the resident carbon pool. As pH declined below 5.3, the dissolution of iron/aluminum (hydr)oxides and corresponding formation of soluble organo-mineral complexes increased the proportion of dissolved organic carbon containing

oxygen-rich compounds (readily oxidizable, labile carbon forms) [51,52]. Additionally, reductive dissolution of iron/manganese oxides could couple with carbon oxidation by Fenton-like reactions [53–55], further increasing SOC lability and loss [24].

On the other hand, the decline in pH likely suppressed the activity of extracellular enzymes involved in the breakdown of complex plant polymers into microbial metabolites, but promoted microbial communities shifting their metabolism toward preferential utilization of simpler substrates [56–58]. Therefore, it resulted in greater MNC accumulation *in situ* rather than its transfer to adjacent particle-size fractions (Figures 7 and 9), that is, slowing the directional flow of plant-derived carbon into MAOC fractions (Figure 3). Additionally, although lowering pH promoted the accumulation and contribution of MNC, it could significantly reduce MAOC pool, because of diminishing the mineral protection (especially Ca^{2+}) of SOC and sorption [28,59,60]. In the absence of mineral protection, MAOC became more susceptible to decomposition into simple organic compounds (e.g., fatty acids), eventually mineralizing to CO_2 [61]. Further, these newly formed simple organics could stimulate the decomposition of native SOC protected by minerals due to priming effect [62].

The apparent contradiction was resolved by distinguishing between chemical lability (an indicator of oxidizability) and physical mobility (an indicator of inter-particle translocation). Acidification enhanced the former by altering mineral-organic interactions, while it constrained the latter by disrupting microbial-mediated carbon processing and organo-mineral association. This decoupling highlighted that soil carbon persistence was not solely governed by its chemical recalcitrance, but was critically dependent on the interplay between pH-modulated microbial physiology and mineral surface chemistry, exhibiting a critical pH threshold.

The observed decoupled C-N dynamics and their sensitivity to management practices and pH was also interpreted within the context of soil texture, particularly the high sand content (60–90%) of the studied Cambisols (Table 1). In sandy soils, the inherent scarcity of fine particles (silt and clay) may have reduced the physical capacity for forming and stabilizing microaggregates and MAOC [63,64], potentially explaining the observed vulnerability of the MAOC pool to acidification-induced mineral dissolution. Additionally, the predominance of sand-sized primary particles and limited binding agents (e.g., Ca^{2+} , Fe/Al oxides) implied that a significant portion of the “aggregates” recovered, especially in the coarse size classes, may represent loose, temporary associations rather than water-stable structural units bound by persistent organic-mineral cements [65,66]. This texture-driven limitation in aggregate hierarchy development could have attenuated the directional flow of plant-derived carbon into finer, more protected fractions, as suggested by the diminished inter-particle carbon transfer under acidification. Therefore, while management practices and pH shift significantly modulated carbon lability and stabilization pathways, the ultimate expression of these processes and the absolute magnitude of carbon sequestration potential may be intrinsically capped by the sandy matrix of these soils. Future investigations in finer-textured soils are warranted to disentangle the interactive effects of texture and management on the mechanisms of C-N decoupling.

Generally, our results demonstrated that the translocation of carbon and nitrogen could not be fully synchronized due to their different pathways through the particle-size matrix, so management strategies could be tailored to independently target carbon sequestration and nitrogen retention, creating a more flexible framework for sustainable soil fertility improvement. However, future research needs to enable direct quantification of carbon and nitrogen translocation fluxes across particle-size fractions using ^{13}C - ^{15}N dual isotopic labeling approaches. Also, it requires determining whether the decoupled C-N dynamics persists over decadal timescales by long-term monitoring.

4.4. Study Limitation and Future Research

While this study has obtained meaningful insights into the decoupling characteristics of carbon–nitrogen cycling and the identification of critical pH threshold between organic and conventional apple orchards, several key limitations must be explicitly acknowledged for a rigorous interpretation of the results.

First, the targeted sampling design was restricted exclusively to apple-planting areas with Cambisol in the Jiaodong Peninsula under a temperate monsoon climate. The geographically constrained scope inherently limited the direct extrapolation of our conclusions to other regions with divergent soil parent materials, climatic regimes, or alternative cropping systems, unless validated through dedicated cross-regional verification. Second, the unbalanced sample size between the two cultivation groups represented an unavoidable methodological limitation. The discrepancy in sample quantity may introduce potential constraints on the statistical robustness of inter-group comparison, and partially restrict the generalizability of findings specific to the organic orchard scenario. Third, this study relied on a space-for-time substitution rather than long-term continuous in situ observation of the same cohort of orchards, which prevented a full capture of the dynamic evolution of carbon–nitrogen coupling relationships across the entire transition cycle from conventional to organic management. Additionally, the absence of measurements on microscopic indicators, including microbial community functional genes and fractionated SOC pools, means that our interpretation of the internal mechanisms underlying C–N cycling decoupling remains confined to the macroscopic element coupling level.

Building on these limitations, future research will be expanded along three core directions. First, we will expand the sampling gradient to cover China's major apple-producing regions, including the Loess Plateau and the entire Bohai Sea Rim, encompassing additional soil types such as Luvisol and Aridisol. This cross-regional sampling framework will allow us to define the explicit applicability boundary of current findings and substantially improve the generalizability of the research results. Second, we will set up a long-term in situ observation platform for organic apple orchards to dynamically monitor the full trajectory of carbon–nitrogen coupling processes and associated microbial succession. Concurrently, we will expand the sample size of organic orchards to balance the quantity ratio between the two groups, further improving the statistical power of comparative analyses. Third, we will integrate multi-technical approaches including metagenomic sequencing, stable isotope tracing, and physical–chemical fractionation of SOC. These methods will enable us to systematically unravel the microbial functional mechanism underlying C–N cycling decoupling and the identified critical pH threshold, clarify the regulatory pathways through which different cultivation systems mediated soil carbon flow direction, and ultimately provide more sufficient theoretical basis for formulating targeted soil quality improvement and nutrient management strategies in organic orchards.

5. Conclusions

This study demonstrated that soil particle-size and management practice exerted distinct and interactive controls on SOC and TN dynamics: particle size dominated $\delta^{13}\text{C}$ variability and drove directional carbon translocation along the particle-size gradient, while management practice primarily regulated $\delta^{15}\text{N}$ distribution and universal N enrichment across all size fractions. This divergent regulatory pattern provided direct evidence for the inherent decoupling of SOC and TN turnover within soil particle-size fractions. A critical pH threshold of ~ 5.3 governed the coupled processes of inter-particle-size carbon flow, MNC accumulation, and SOC stabilization. At this threshold, inter-particle-size carbon translocation reached its maximum while microbial decomposition was minimized, offering a clear, actionable target for pH modulation to optimize agricultural carbon sequestration.

Organic management reshaped particle-size carbon turnover pathways by suppressing plant-derived carbon translocation among fractions, while substantially boosting microbial anabolism. This shift drastically elevated microbial-derived carbon stocks in both POC and MAOC pools, ultimately promoting more persistent long-term SOC sequestration relative to conventional management. The counterintuitive co-occurrence of elevated SOC chemical lability and enhanced SOC stabilization under progressive soil acidification revealed an unreported decoupling mechanism: intensified carbon translocation driven by rising chemical oxidizability increased SOC lability, while accumulated MNC derived from stimulated microbial anabolism and physical protection concurrently promoted SOC stabilization.

These findings closed the knowledge gap regarding C-N coupling within soil particle-size fractions under contrasting orchard management regimes, and provided theoretical support for the sustainable optimization of SOC and TN pools in perennial fruit agroecosystems.

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Abbreviations

The following abbreviations are used in this manuscript:

SOC	Soil organic carbon
TN	Total nitrogen
MNC	Microbial necromass carbon
FNC	Fungal necromass carbon
BNC	Bacterial necromass carbon
POC	Particulate organic carbon
MAOC	Mineral-associated organic carbon
PTN	Particulate-associated total nitrogen
MATN	Mineral-associated total nitrogen
VLC	Very labile carbon
LC	Labile carbon
LLC	Less labile carbon
NLC	Non-labile carbon
L	Lability
LI	Lability index
AS	Amino sugar
Glu	Glucosamine
Mur	Muramic acid
ANOVA	Analysis of variance

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