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Research Article

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Fungi dominate microbial necromass while bacteria dominate living biomass in tea plantation soils

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Abstract

Background and Aims: Fungal and bacterial contributions to soil organic carbon (SOC) depend on both biomass production and the fate of their residues, so fungal–bacterial dominance in living communities may differ from that in accumulated necromass. We examined biomass–necromass relationships and their associations with SOC quantity and chemical composition in strongly acidic tea plantation soils.

Methods: Soils (0–30 cm) were sampled from four *Camellia sinensis* (L.) Kuntze varieties cultivated for approximately 19 years. We measured SOC and phospholipid fatty acids (PLFAs), estimated necromass C from amino sugars, and characterized SOC chemistry using solid-state ^{13}C nuclear magnetic resonance spectroscopy.

Results: Soils planted with ‘Fuding Dabaicha’ (FD) had the highest SOC content and stock and the highest alkyl C/O-alkyl C ratio. Bacteria accounted for 61–67% of total PLFAs and fungal markers for only 9–10%, whereas fungal residues represented approximately 89–93% of estimated microbial necromass C (MNC). MNC accounted for approximately 45.4 – 71.2% of SOC, whereas lignin-phenol-derived C (PDC) accounted for 3.6–7.3%. Total, fungal, and bacterial PLFAs were positively associated with their corresponding necromass C pools across plots. Redundancy analysis was significant, with SOC and microbial necromass oriented toward positive RDA1 scores and PDC, O-alkyl C, and lignin acid-to-aldehyde ratios toward negative scores.

Conclusion: Living microbial biomass and accumulated necromass were positively related in quantity but strongly contrasted in bacterial–fungal composition under acidic tea plantation conditions. This contrast highlights post-mortem processing and residue preservation as links between contemporary microbial communities and SOC pools.

Keywords: tea variety; soil organic carbon; microbial necromass; phospholipid fatty acids; amino sugars

Introduction

Soil organic carbon (SOC) is central to the global carbon cycle, climate-change mitigation, soil fertility, and ecosystem resilience (Bossio et al. 2020; Lal 2004; Lehmann et al. 2020). Microorganisms contribute to SOC through living biomass and residues that remain after cell death. Although living microbial biomass is a small and transient SOC fraction, microbial necromass can accumulate as a substantial component of soil C pools (Liang and Balser 2011; Liang et al. 2017, 2019; Miltner et al. 2009; Wang et al. 2021). These pools represent different temporal dimensions of microbial C cycling. Phospholipid fatty acids (PLFAs) reflect contemporary microbial biomass, whereas amino-sugar-derived necromass integrates microbial growth, mortality, decomposition, recycling, and preservation over longer periods (Camenzind et al. 2023; Shao et al. 2019; Zheng et al. 2021). It therefore remains unclear whether variation in living biomass is translated into necromass accumulation and whether the bacterial–fungal composition of the living community persists in the accumulated residue pool.

This question is especially relevant to fungi and bacteria because their living biomass and residues can follow different trajectories after cell death. Fungal and bacterial residues differ in cell-wall chemistry, decomposability, microbial reuse, and interactions with mineral surfaces (Angst et al. 2021; Hu et al. 2020). Fungal cell-wall materials, including chitin-derived compounds, may persist through relatively slow decomposition and organo-mineral association. By contrast, bacterial cell-wall components such as peptidoglycan can be recycled rapidly by living microorganisms. Consequently, bacterial dominance in contemporary biomass does not necessarily imply bacterial dominance in accumulated necromass. Distinguishing the strength of biomass–necromass relationships from the composition of the two pools may clarify how living microbial communities are translated into longer-lived residues.

Strongly acidic soils provide an important setting in which to examine these relationships because acidity can influence microbial physiology, community composition, residue decomposition, and mineral–organic interactions (Hu et al. 2020; Malik et al. 2018). Tea plantations are particularly suitable for this purpose. Their soils are commonly acidic, while perennial roots, frequent pruning, and residue return continuously supply organic C. Some inputs may remain recognizable as plant-derived C (PDC), whereas others are assimilated by microorganisms and subsequently enter microbial necromass pools. Lignin phenols and amino sugars therefore provide complementary indicators of plant- and microbial-derived C, respectively (Angst et al. 2021; Yang et al. 2022). Solid-state ^{13}C nuclear magnetic resonance (NMR)

spectroscopy can also characterize SOC chemical composition from the relative abundances of alkyl C, O-alkyl C, aromatic C, and carbonyl C (Adnan et al. 2021; Baldock et al. 1992; Dou et al. 2008). An integrated assessment of living biomass, necromass, PDC, and SOC chemistry can thus clarify C accumulation and transformation in acidic tea plantation soils.

Previous work at this site showed that soils of different tea varieties vary markedly in SOC stocks and chemical composition (Du et al. 2023). We examined soils of four tea varieties cultivated for approximately 19 years: *Chuancha No. 3* (CC3), *Chuanmu No. 217* (CM217), *Chuannong Huangyazao* (CN), and *Camellia sinensis* ‘*Fuding Dabaicha*’ (FD). We combined SOC measurements, solid-state ¹³C NMR spectroscopy, PLFA profiling, amino-sugar biomarkers, and lignin phenol analysis. Our objectives were to characterize SOC quantity and chemistry, quantify living microbial and necromass pools, and test their quantitative and compositional correspondence. We specifically asked whether total, fungal, and bacterial PLFAs were associated with MNC, FNC, and BNC, respectively. We then assessed whether bacterial–fungal dominance in living biomass was retained in accumulated necromass under strongly acidic conditions.

Materials and methods

Study area and tea variety descriptions

The study was conducted in a long-term tea variety comparison plantation in the hilly region of western Sichuan, China. The region has a subtropical monsoon climate, with a mean annual temperature of approximately 15.4 °C and annual precipitation of approximately 1632.8 mm. The clay-loam soil is classified as a Luvisol according to the World Reference Base for Soil Resources (IUSS Working Group 2014).

The experimental plantation covers approximately 900 m² and was used as a tea seedling nursery before the variety comparison trial was established in 2005. Four varieties of *Camellia sinensis* (L.) Kuntze were included: ‘Fuding Dabaicha’ (FD), Chuannong Huangyazao (CN), Chuanmu No. 217 (CM217), and Chuancha No. 3 (CC3). Each variety was represented by three spatially distinct field plots, giving 12 plot-level experimental units. Individual plots covered approximately 28–35 m².

FD is a nationally recognized variety widely grown in the low-mountain and hilly regions of western Sichuan. CN, CM217, and CC3 are provincially selected varieties adapted to the local environment. CN flushes approximately 8–10 days earlier than FD and produces an approximately 10% greater yield. CC3

has relatively strong drought and cold resistance. CC3 and CN are shrub-type varieties with plant heights of approximately 60 cm. CM217 and FD are semi-arborescent varieties with heights of approximately 80 and 70 cm, respectively. The canopy widths of CC3, CM217, CN, and FD were approximately 85, 100, 90, and 110 cm, respectively.

Tea plants were grown at approximately 8×10^4 plants ha^{-1} in alternating wide and narrow rows. The corresponding row spacings were 1.50 ± 0.04 m and 0.30 ± 0.15 m. Management was consistent among plots. Major pruning was usually conducted in spring (March–April), with lighter pruning in autumn (approximately October); all shoots and leaves were retained in situ. A tea-specific organic fertilizer was applied in November at 15 t ha^{-1} . Compound fertilizer was applied as top dressing from April to June at $600\text{--}750 \text{ kg ha}^{-1}$. Fertilizers were placed in furrows along the canopy margin at depths of approximately 25 cm for organic fertilizer and 15 cm for compound fertilizer, followed by immediate backfilling.

Soil sampling and analyses

Soil sampling

Soils were sampled in April 2024 after spring pruning. Within each of the 12 plots, three cores were collected randomly from 0–30 cm depth and composited into one representative plot-level sample. The cores were subsamples, and each field plot was the statistical unit ($n = 3$ plots per variety; 12 composite samples total). After visible roots and stones were removed, each composite sample was divided into two portions. One portion was passed through a 2-mm sieve, homogenized, and stored at 4°C for phospholipid fatty acid (PLFA) analysis. The other was air-dried, finely ground, and passed through a 0.125-mm sieve for SOC, total nitrogen (TN), amino sugar, lignin phenol, and solid-state ^{13}C NMR analyses.

Soil pH was measured using a glass electrode in a soil–water suspension. Bulk density (BD) was measured with a 100- cm^3 soil core sampler. SOC and TN contents were measured with an Elementar Vario Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Germany). The soil C/N ratio was calculated from these values. SOC stock was calculated as follows:

$$SOC \text{ stock} = \frac{SOC \times BD \times H}{100}$$

where SOC is the carbon content (g kg^{-1}), BD is bulk density (g cm^{-3}), and H is sampling depth (cm).

Amino sugar analysis

Amino sugars were quantified by Chengdu Baihui Biotechnology Co., Ltd. using an acid-hydrolysis method adapted from Zhang and Amelung (1996). Finely ground soil containing approximately 0.3 mg N was hydrolyzed with 10 mL of 6 mol L⁻¹ HCl at 105 °C for 8 h. After cooling, the solution was filtered and 100 µL of myo-inositol was added as an internal standard. The hydrolysate was evaporated to dryness, redissolved in water, and adjusted to pH 6.6–6.8. After centrifugation, the supernatant was freeze-dried and derivatized with hydroxylamine hydrochloride and 4-(dimethylamino)pyridine in pyridine–methanol. Derivatives were analyzed by gas chromatography (Agilent 6890A) with flame-ionization detection and a DB-1 fused-silica column. Amino sugar concentrations were calculated by the internal-standard method. Microbial necromass carbon (MNC) was estimated from glucosamine (GluN) and muramic acid (MurA) using the following empirical equations (Hu et al. 2024):

$$FNC = (GluN - 1.16 \times MurA) \times 10.8$$

$$BNC = MurA \times 31.3$$

$$MNC = FNC + BNC$$

where FNC and BNC are fungal and bacterial necromass C, respectively (g kg⁻¹), and GluN and MurA are glucosamine and muramic acid contents (g kg⁻¹). All unit conversions were completed before applying the equations. As a calculation check, MNC was independently recalculated using an alternative published conversion procedure after harmonizing units; the among-soil pattern and resulting interpretation were unchanged.

Lignin phenol analysis

Lignin phenols were quantified by Chengdu Baihui Biotechnology Co., Ltd. using alkaline CuO oxidation adapted from Feng and Simpson (2007). Soil samples were milled and passed through a 0.149-mm sieve. A C18-based separation procedure adapted from Hatfield and Fukushima (2005) was followed by derivatization with pyridine and N,O-bis(trimethylsilyl)trifluoroacetamide. The mobile phase was modified to improve phenolic resolution. Phenolic compounds were separated and quantified by gas chromatography (Agilent 6890A) with flame-ionization detection. Lignin phenols were expressed as the sums of vanillyl (V), syringyl (S), and cinnamyl (C) units. Acid-to-aldehyde ratios for S and V units, (Ad/Al)S and (Ad/Al)V, were used as indicators of lignin transformation. Plant-derived carbon (PDC)

was estimated from lignin phenols following Yang et al. (2022).

$$\text{Plant-derived carbon} = \frac{(\frac{V_p}{0.333} + \frac{S_p}{0.90} + C_p)}{SOC \times 3\%}$$

where V, S, C, and SOC are expressed in g kg⁻¹. The terms 0.333 and 0.90 correspond to the published conversion/recovery terms for V- and S-type phenols, respectively, and 0.03 represents the assumed minimum lignin content of the principal plant residues (Yang et al. 2022).

Phospholipid fatty acid analysis

Phospholipid fatty acid analysis followed Frostegård and Bååth (1996). Phospholipids were extracted from 2.0 g of soil and quantified. Fatty acid methyl esters were identified by gas chromatography (Agilent 6890A) with an Agilent 19091B-102 column. Bacterial biomass was represented by biomarkers including i14:0, i15:0, a15:0, and i16:0, and fungal biomass by 18:1ω9c and 18:2ω6c (Table S1; Smith et al. 2015). PLFA contents are reported as µg g⁻¹ soil.

Solid-state ¹³C cross-polarization/magic-angle-spinning NMR analysis

Approximately 5.0 g of soil was pretreated with 10% hydrofluoric acid for eight cycles, washed, and oven-dried. Solid-state ¹³C cross-polarization/magic-angle-spinning NMR spectra were acquired with a Bruker Avance III 500-MHz spectrometer. Chemical shifts were assigned to alkyl C (0–45 ppm), O-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carbonyl C (160–220 ppm). SOC composition was expressed as the relative proportion of each spectral region. The humification index (HIX) was calculated as follows:

$$\text{Humification index} = \frac{\text{alkyl C}}{O - \text{alkyl C}}$$

Statistical analyses

Statistical analyses were performed in SPSS version 27.0 and R. The field plot was the statistical unit, giving three independent plots per variety (n = 3; 12 plots total). Differences among soils of the tea varieties were evaluated by one-way analysis of variance (ANOVA). When the ANOVA was significant, means were compared using Fisher's least significant difference test at P < 0.05. Simple linear regression tested associations between total microbial PLFA and MNC, fungal PLFA and FNC, and bacterial PLFA and BNC across the 12 plots; these relationships were interpreted as associations rather than causal effects. Redundancy analysis (RDA) was used as an exploratory multivariate ordination to summarize

coordinated variation in SOC, microbial, plant-derived, and SOC chemical indicators among soils planted with the four tea varieties. The analysis included 18 z-standardized indicators: SOC; fungal, bacterial, and total microbial necromass C (FNC, BNC, and MNC); plant-derived C (PDC); total, bacterial, and fungal PLFAs; vanillyl, syringyl, and cinnamyl phenols; (Ad/Al)V and (Ad/Al)S; alkyl C, O-alkyl C, aromatic C, carbonyl C, and HIX. Tea variety was used as the constraining factor, and significance of the global model and constrained axes was evaluated using 9,999 unrestricted permutations. Because several variables are mathematically or biologically related, the RDA was used only to visualize joint multivariate covariation and was not interpreted as evidence of independent effects or causal relationships.

Results

SOC content and stock

SOC content and stock differed significantly among soils of the four tea varieties (Fig. 1). FD had the highest SOC content (24.72 g kg^{-1}), followed by CN (19.98 g kg^{-1}). CM217 and CC3 had lower SOC contents than FD and CN (Fig. 1A). SOC stock showed a similar pattern. FD had the highest stock (9.42 kg m^{-2}), whereas CC3 had the lowest (5.38 kg m^{-2} ; Fig. 1B).

SOC chemical composition

O-alkyl C was the largest SOC fraction in every soil, ranging from 42.43% to 48.53% (Table 1). CC3 had the highest O-alkyl C proportion, whereas FD had a lower proportion than CN, CM217, and CC3. FD also had the highest humification index (HIX), indicating a more extensively transformed SOC chemical composition.

Soil amino sugars

Glucosamine (GluN) was the predominant amino sugar. FD had the highest GluN and muramic acid (MurA) contents, whereas CC3 had the lowest (Fig. 2A, D). Total amino sugar content decreased in the order $\text{FD} (1.81 \text{ g kg}^{-1}) > \text{CN} (1.33 \text{ g kg}^{-1}) > \text{CM217} (0.83 \text{ g kg}^{-1})$ and $\text{CC3} (0.79 \text{ g kg}^{-1})$ (Fig. 2E).

Soil lignin phenols

Lignin-derived phenol contents varied little among soils of the tea varieties (Fig. 3A–D). Vanillyl, syringyl, cinnamyl, and total lignin phenol contents did not differ significantly. The acid-to-aldehyde ratios, however, differed among varieties. (Ad/Al)S was lowest in FD and highest in CN, whereas

(Ad/Al)V was lowest in FD and highest in CC3 (Fig. 3E, F). These ratios indicate variety-associated differences in lignin transformation despite similar total lignin phenol contents.

Accumulation and contribution of MNC, PDC, and living microbial biomass

Fungal necromass C (FNC) was the principal component of MNC in every soil and ranged from approximately 7.00 to 16.13 g kg⁻¹ (Fig. 4A). FNC contributed 42.4–65.3% of SOC, whereas BNC contributed only 3.0–6.8% (Fig. 4B). Consequently, MNC accounted for approximately 45.4–71.2% of SOC across the four soils. PDC contents were much smaller (approximately 0.9–1.1 g kg⁻¹), did not differ significantly among the four soils, and represented approximately 3.6–7.3% of SOC.

Living microbial biomass showed a different composition (Fig. 4C, D). Total PLFA contents were 28.29, 28.43, 21.19, and 12.85 µg g⁻¹ in FD, CN, CM217, and CC3, respectively. Bacterial PLFAs were the largest identifiable biomass component in every soil, accounting for approximately 60–67% of total PLFA. Fungal PLFAs accounted for approximately 9–10%.

Despite bacterial dominance in living biomass, accumulated necromass showed the opposite pattern. Fungal residues represented approximately 89–93% of MNC, with FNC/BNC ratios of approximately 8.5–14.1, whereas fungal/bacterial PLFA ratios were only 0.14–0.16 (Fig. 4B, D). Thus, bacteria dominated contemporary microbial biomass, whereas fungi dominated the accumulated microbial residue pool.

Quantitative relationships between living microbial biomass and necromass

Microbial biomass was positively associated with the corresponding necromass pools across the 12 plots (Fig. 5). Total microbial PLFA was associated with MNC ($R^2 = 0.5984$, $P < 0.01$), fungal PLFA with FNC ($R^2 = 0.6166$, $P < 0.01$), and bacterial PLFA with BNC ($R^2 = 0.4854$, $P < 0.05$).

Multivariate associations among SOC, microbial residues, and soil C characteristics

The global RDA model showed significant multivariate differentiation among soils planted with the four tea varieties (pseudo- $F = 5.231$, $P = 0.0001$, $R^2 = 0.662$, adjusted $R^2 = 0.536$; Fig. 6). RDA1 and RDA2 accounted for 49.9% and 8.7% of the total standardized multivariate variance, respectively. RDA1 was significant ($P = 0.0002$), whereas RDA2 and RDA3 were not significant (both $P = 0.0624$). Along RDA1, SOC, FNC, BNC, MNC, HIX, and total, fungal, and bacterial PLFAs were oriented toward positive scores, whereas PDC, O-alkyl C, (Ad/Al)V, and (Ad/Al)S were oriented toward negative scores. FD and

CC3 plots occurred mainly on opposite sides of RDA1, while CN and CM217 occupied intermediate regions of the ordination space.

Discussion

Differences in SOC quantity are accompanied by changes in chemical composition

SOC content and stock differed substantially among soils of the four tea varieties. Previous work at the same site also linked long-term cultivation of different tea varieties to variation in aggregate-associated C pools and SOC characteristics (Du et al. 2023). The agreement supports the persistence of variety-associated SOC patterns at this site. Our ^{13}C NMR results further show that differences in SOC quantity were accompanied by systematic differences in SOC chemical composition.

O-alkyl C dominated the spectra of all four soils, consistent with agricultural soils in which carbohydrates and other O-substituted compounds are major components of plant and microbial inputs (Audette et al. 2021; Baldock et al. 1992; Boeni et al. 2014). Its relative abundance nevertheless varied markedly among varieties. FD had the lowest O-alkyl C proportion and the highest HIX, whereas CC3 showed the opposite pattern. O-alkyl C largely comprises carbohydrate-like structures that are preferentially used during decomposition. The relative contribution of alkyl C commonly increases as decomposition proceeds (Audette et al. 2021; Baldock et al. 1992; Helfrich et al. 2006). Comparable shifts have occurred under long-term agricultural management and indicate changes in the degree of SOC transformation (Li et al. 2015; Luan et al. 2019).

Alkyl C itself did not differ significantly among the four soils. The higher HIX in FD therefore resulted mainly from its lower relative abundance of O-alkyl C rather than an absolute enrichment of alkyl C. FD also had a higher carbonyl C proportion. Carbonyl C includes carboxyl, amide, and other oxidized structures that may become relatively enriched during microbial and oxidative transformation (Baldock et al. 1992; Dou et al. 2008). Together, the lower O-alkyl C, higher HIX, and greater carbonyl C proportion indicate more extensive chemical transformation of SOC in FD.

These NMR characteristics do not directly demonstrate that a particular SOC pool is intrinsically resistant to decomposition or has a longer residence time. SOC persistence depends on substrate accessibility, microbial activity, aggregation, and mineral interactions, not molecular recalcitrance alone (Cotrufo et al. 2019; Lehmann and Kleber 2015; Schmidt et al. 2011). Our NMR data therefore indicate differences in chemical transformation. The biological origin and persistence of this C must be interpreted together

with plant and microbial biomarkers.

Bacterial dominance in living biomass contrasts with fungal dominance in accumulated necromass

Plant- and microbial-derived C showed strongly contrasting estimated contributions to SOC. Lignin-phenol-derived PDC accounted for approximately 3.6–7.3% of SOC, whereas MNC accounted for approximately 45.4–71.2%. The smaller biomarker-defined PDC fraction does not imply low plant-C input, because tea plantations receive continuous root inputs and regular return of pruning residues. Rather, lignin phenols quantify a recognizable subset of plant-derived C. Plant substrates can also be decomposed, assimilated into microbial biomass, and subsequently incorporated into SOC as residues after microbial death (Cotrufo et al. 2013; Kallenbach et al. 2016; Liang et al. 2017). Thus, the contrast between biomarker-defined PDC and the larger MNC pool is consistent with substantial microbial processing of plant inputs, but it should not be interpreted as a complete partitioning of all plant- and microbial-derived SOC.

The large MNC contribution is consistent with evidence that microbial residues are major components of SOC. A global synthesis estimated mean MNC contributions of 51% in croplands, 47% in grasslands, and 35% in forests, with more than 65% of microbial necromass being fungal-derived (Wang et al. 2021). Liang et al. (2019) likewise concluded that microbial necromass can comprise more than half of SOC in temperate topsoils. In western Sichuan tea plantations, MNC contributed 30.8–50.5% of SOC and PDC contributed 11.2–27.8% (Xu et al. 2026). Our MNC estimates extend to a higher upper range, whereas the lignin-phenol-derived PDC fractions are lower. These cross-study differences should be interpreted cautiously because soil properties, plant inputs, management, analytical units, and biomarker conversion procedures differ among studies. To test whether our interpretation depended on one specific calculation form, we independently recalculated both MNC and PDC using alternative published formulations after harmonizing units and conversion steps. The recalculations produced the same among-soil pattern and the same overall conclusion that MNC greatly exceeded biomarker-defined PDC. Thus, the main contrast was unchanged by the calculation form used, although absolute pool sizes remain biomarker-based estimates.

The fungal dominance of MNC was more striking than its total size. Fungal residues represented approximately 89–93% of MNC, well above the global average of more than 65% reported by Wang et

al. (2021). That synthesis partly attributed fungal necromass dominance to greater living fungal than bacterial biomass. Our soils showed the opposite living-community pattern: bacterial PLFAs exceeded fungal PLFAs in every variety (Table S2). Greater contemporary fungal biomass therefore cannot explain the strong fungal signature in accumulated necromass. This consistent contrast provides evidence that the composition of living microbial biomass is decoupled from that of accumulated residues.

This decoupling partly reflects the different time scales represented by PLFAs and necromass. PLFAs primarily indicate contemporary living biomass, whereas necromass integrates repeated microbial growth, death, decomposition, reuse, and preservation. Microbial death also does not transfer living-cell composition unchanged into the residue pool. Death pathways alter residue chemistry and persistence, and subsequent decomposition and stabilization further reshape the remaining material (Camenzind et al. 2023). Differences in residue fate can amplify this divergence. Bacterial peptidoglycan can be decomposed and reused efficiently, whereas fungal cell-wall compounds such as chitin follow different decomposition trajectories (Hu et al. 2020). Mineral association further affects retention (Angst et al. 2021; Cotrufo et al. 2019). These processes can allow a relatively small standing fungal biomass to leave a disproportionately large necromass pool.

Strong acidity provides an important environmental context for this pattern. Soil pH ranged from 4.56 to 4.86 (Table S3), close to the national mean of 4.68 reported for Chinese tea plantations (Yan et al. 2020). Bacteria generally respond more strongly than fungi to declining pH, and lower pH has been associated with greater microbial necromass accumulation (Rousk et al. 2010; Wang et al. 2021). Bacteria nevertheless dominated living PLFAs in every soil studied here. Fungal dominance of necromass therefore cannot be attributed simply to fungal dominance in living biomass under acidic conditions. Acidity may instead influence growth, residue decomposition, reuse, and organo-mineral interactions. Because we did not include neutral or weakly acidic soils, the study cannot identify acidity as the cause of decoupling. It shows that compositional decoupling occurs consistently under strongly acidic tea plantation conditions.

Quantitative biomass–necromass relationships and implications for SOC

Although living biomass and necromass were compositionally decoupled, their pool sizes were positively associated. Total, fungal, and bacterial PLFAs were each related to their corresponding necromass pools. These relationships are consistent with living microbial biomass providing the biological precursor from

which necromass is ultimately formed, while remaining cross-sectional associations rather than direct measurements of turnover.

The coexistence of quantitative coupling and compositional divergence is central to our interpretation. A larger living microbial pool can provide more material for necromass formation, consistent with the relationships in Fig. 5. Mortality, decomposition, reuse, and physicochemical stabilization then modify the relative preservation of fungal and bacterial residues (Camenzind et al. 2023; Hu et al. 2020). Living biomass and necromass can therefore be coupled in pool size while remaining decoupled in bacterial–fungal dominance.

The RDA provides a broader multivariate context for the pairwise biomass–necromass relationships. The global model was significant and explained 66.2% of the total standardized multivariate variation, with an adjusted R^2 of 0.536. Because only RDA1 was statistically significant, interpretation should focus primarily on this axis. SOC, FNC, BNC, MNC, and the total, fungal, and bacterial PLFA vectors were all directed toward positive RDA1 scores. This common directional component is consistent with the positive PLFA–necromass relationships shown in Fig. 5 and indicates that larger living microbial pools, larger accumulated necromass pools, and greater SOC tended to covary. At the same time, the RDA shows that this common microbial gradient was accompanied by contrasting variation in plant-derived and SOC chemical indicators. PDC, O-alkyl C, and both lignin acid-to-aldehyde ratios were oriented toward negative RDA1 scores, whereas HIX was oriented toward positive RDA1 scores. This configuration is consistent with the coexistence of greater microbial residue accumulation and a more extensively transformed SOC chemical composition toward the positive side of RDA1. The opposite orientation of PDC and MNC further indicates that the biomarker-defined plant-derived and microbial-derived C pools did not vary proportionally among the four soils. However, the RDA vectors must be interpreted jointly rather than as independent effects because MNC is derived from FNC and BNC, total PLFA contains bacterial and fungal PLFAs, HIX is derived from alkyl C and O-alkyl C, and PDC is calculated from lignin-phenol biomarkers. The ordination therefore summarizes coordinated multivariate structure rather than identifying independent drivers of SOC accumulation.

The large MNC pool also has implications for SOC, but this relationship requires caution. MNC accounted for approximately 45.4–71.2% of SOC, confirming that microbial residues were a major component of the total C pool. However, MNC is estimated as a component of SOC. Any statistical

relationship between MNC and SOC would therefore contain an inherent part–whole association and could not establish MNC as an independent driver of SOC accumulation. We consequently emphasize associations between PLFAs and corresponding necromass pools, together with the descriptive RDA ordination, rather than using MNC to predict SOC.

After microbial residues form, their persistence depends on mineral association, aggregate protection, spatial accessibility, and continued microbial processing (Cotrufo et al. 2019; Schmidt et al. 2011). Previous work at this site showed that aggregate-associated C pools contributed substantially to SOC differences among soils of different tea varieties (Du et al. 2023). This finding provides a physicochemical context for our results. Necromass formation supplies organic material to SOC, but its long-term retention depends on interactions between residues and the soil matrix. Variation in NMR-derived composition likely reflects both microbial processing and physicochemical preservation rather than necromass content alone.

Overall, our results identify two complementary relationships between living microorganisms and necromass in acidic tea plantation soils. Living biomass was positively associated with the corresponding necromass pool, but bacterial–fungal dominance differed sharply between living biomass and accumulated residues. The RDA further placed SOC and microbial necromass along a common multivariate gradient while separating them from O-alkyl C and lignin-transformation characteristics. Together, these findings link contemporary microbial communities with longer-term residue accumulation without implying a single causal pathway. SOC integrates plant inputs, microbial growth and death, residue transformation, and physicochemical preservation.

Conclusions

Living microbial biomass and accumulated necromass were positively related in quantity but contrasted strongly in bacterial–fungal composition in strongly acidic tea plantation soils. Total, fungal, and bacterial living biomass were each positively associated with the corresponding necromass pool. Bacteria dominated living biomass, whereas fungal residues represented approximately 89–93% of MNC. MNC accounted for approximately 45.4–71.2% of SOC, while lignin-phenol-derived PDC represented a smaller biomarker-defined fraction of approximately 3.6–7.3%. The RDA further identified a significant multivariate gradient in which SOC and microbial necromass occurred on the positive side of RDA1 together with living microbial PLFAs and HIX, whereas PDC, O-alkyl C, and lignin acid-to-aldehyde

ratios were oriented toward the negative side. Together with the ^{13}C NMR results, these patterns indicate coordinated variation among living microbial biomass, accumulated residues, and SOC chemical composition. Contemporary microbial community composition alone is therefore insufficient to predict the biological composition of accumulated residues.

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Statements and Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

Tao Liu: Conceptualization, supervision, writing – original draft, and writing – review and editing. Xizhou Zhang: Conceptualization, investigation, and supervision. Jingchao Zhou: Data curation, formal analysis, investigation, visualization, and writing – original draft. Tingxuan Li: Conceptualization, project administration, supervision, and writing – review and editing. Yongdong Wang: Investigation and supervision. Huagang Huang: Funding acquisition and project administration. Haiying Yu: Funding acquisition and project administration. Yu Tang: Software. Keji Wang: Writing – review and editing. Xin Song: Data curation and methodology.

Data availability

The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Table 1 Relative abundance of SOC functional groups and humification index in soils planted with different tea varieties.

Tea variety	Alkyl C (%)	O-alkyl C (%)	Aromatic C (%)	Carbonyl C (%)	Humification index
FD	27.87 ± 0.70 a	42.43 ± 0.32 c	18.15 ± 0.33 a	11.54 ± 0.15 a	0.66 ± 0.02 a
CN	26.99 ± 0.38 a	45.51 ± 0.98 b	18.27 ± 1.25 a	9.23 ± 0.26 c	0.59 ± 0.00 b
CM217	26.68 ± 0.30 a	45.51 ± 0.11 b	17.88 ± 0.31 a	9.92 ± 0.16 b	0.59 ± 0.01 b
CC3	27.65 ± 0.69 a	48.53 ± 0.42 a	15.03 ± 0.65 b	8.79 ± 0.08 c	0.57 ± 0.02 b

Note: Values are means $\pm$ SE (n = 3 independent field plots). Different letters within a column indicate significant differences at P < 0.05 according to Fisher's LSD test.

Fig. 1 SOC content (A) and stock (B) of soils planted with different tea varieties. Note: Different letters indicate significant differences ($p < 0.05$) among the different tea varieties. (FD) *C. sinensis* 'Fuding Dabaicha', (CN) *Chuannong Huangyazao*, (CM217) *Chuanmu No.217*, (CC3) *Chuancha No.3*.

Fig. 2 Amino sugar contents of soils planted with different tea varieties. Note: Glucosamine (A), mannosamine (B), galactosamine (C), muramic acid (D), total amino sugar (E). Note: Different letters indicate significant differences ($p < 0.05$) among the different tea varieties. (FD) *C. sinensis* 'Fuding Dabaicha', (CN) *Chuannong Huangyazao*, (CM217) *Chuanmu No.217*, (CC3) *Chuancha No.3*.

Fig. 3 Lignin phenol contents and acid-to-aldehyde ratios of soils planted with different tea varieties. Note: (A) syringyl phenols (S), (B) vanillyl phenols (V), (C) cinnamyl phenols (C), (D) total lignin phenols, (E) acid-to-aldehyde ratio of syringyl phenols [(Ad/Al)S], and (F) acid-to-aldehyde ratio of vanillyl phenols [(Ad/Al)V]. Note: Different letters indicate significant differences ($p < 0.05$) among the different tea varieties. The acid-to-aldehyde ratio in S- and V-units [(Ad/Al)S and (Ad/Al)V] was employed to evaluate the extent of lignin biotransformation. (FD) *C. sinensis* 'Fuding Dabaicha', (CN) *Chuannong Huangyazao*, (CM217) *Chuanmu No.217*, (CC3) *Chuancha No.3*.

Fig. 4. Distributions of estimated SOC source pools and living microbial biomass in soils planted with different tea varieties. Note: (A) Contents of fungal necromass C (FNC), bacterial necromass C (BNC), and plant-derived C (PDC); (B) proportional contributions of FNC, BNC, PDC, and other C to SOC; (C) contents of fungal, bacterial, nonspecific, and total microbial PLFAs; and (D) proportional composition of fungal, bacterial, and nonspecific microbial PLFAs. Different lowercase letters indicate significant differences in the same component among soils planted with different tea varieties at $P < 0.05$, whereas different uppercase letters indicate significant differences in total PLFA content. (FD) *C. sinensis* 'Fuding Dabaicha', (CN) *Chuannong Huangyazao*, (CM217) *Chuanmu No.217*, (CC3) *Chuancha No.3*.

Fig. 5. Relationships between living microbial biomass and corresponding microbial necromass pools. Note: Relationships are shown between (A) total microbial PLFA and microbial necromass C (MNC), (B) fungal PLFA and fungal necromass C (FNC), and (C) bacterial PLFA and bacterial necromass C (BNC). (FD) *C. sinensis* 'Fuding Dabaicha', (CN) *Chuannong Huangyazao*, (CM217) *Chuanmu No.217*, (CC3) *Chuancha No.3*.

Fig. 6 Redundancy analysis (RDA) of 18 z-standardized SOC, microbial, plant-derived, lignin-phenol, and SOC chemical indicators across the 12 field plots. Note: Tea variety was used as the constraining factor. The global model was significant (pseudo-F = 5.231, $P = 0.0001$, $R^2 = 0.662$, adjusted $R^2 = 0.536$; 9,999 unrestricted permutations). RDA1 and RDA2 accounted for 49.9% and 8.7% of total standardized multivariate variance, respectively. Vectors represent SOC; fungal, bacterial, and total microbial necromass C (FNC, BNC, and MNC); plant-derived C (PDC); total, fungal, and bacterial PLFAs; vanillyl, syringyl, and cinnamyl phenols;

(Ad/Al)V and (Ad/Al)S; alkyl C, O-alkyl C, aromatic C, carbonyl C, and HIX. (FD) *C. sinensis* 'Fuding Dabaicha', (CN) *Chuannong Huangyazao*, (CM217) *Chuanmu No.217*, (CC3) *Chuancha No.3*.

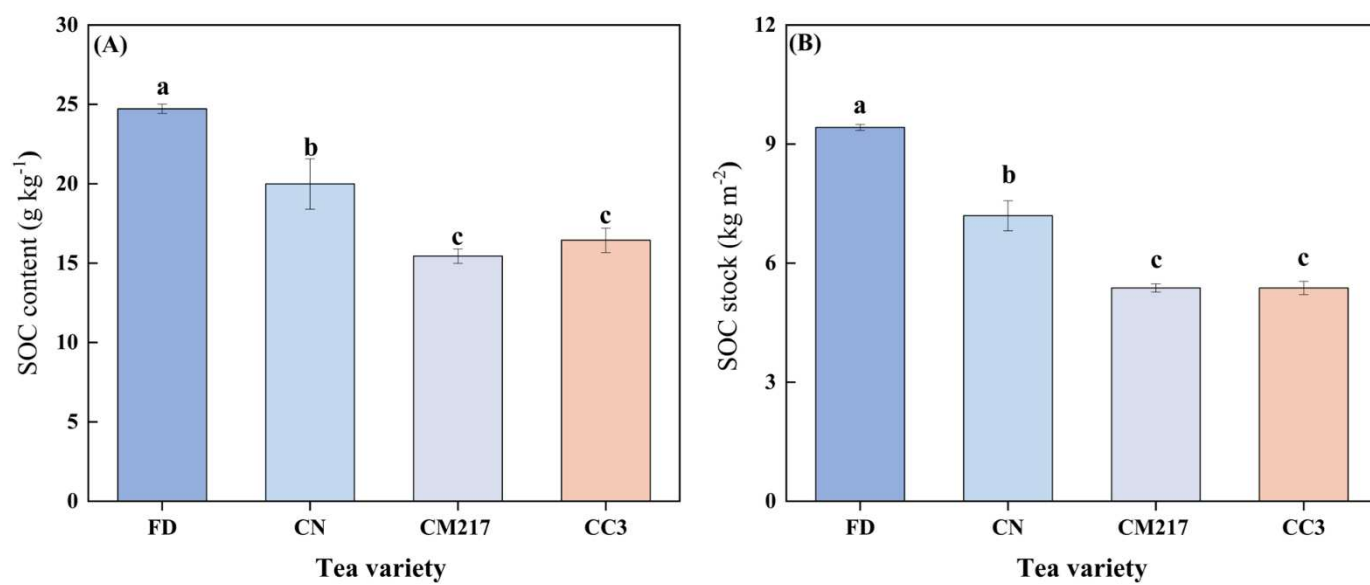


Fig. 1

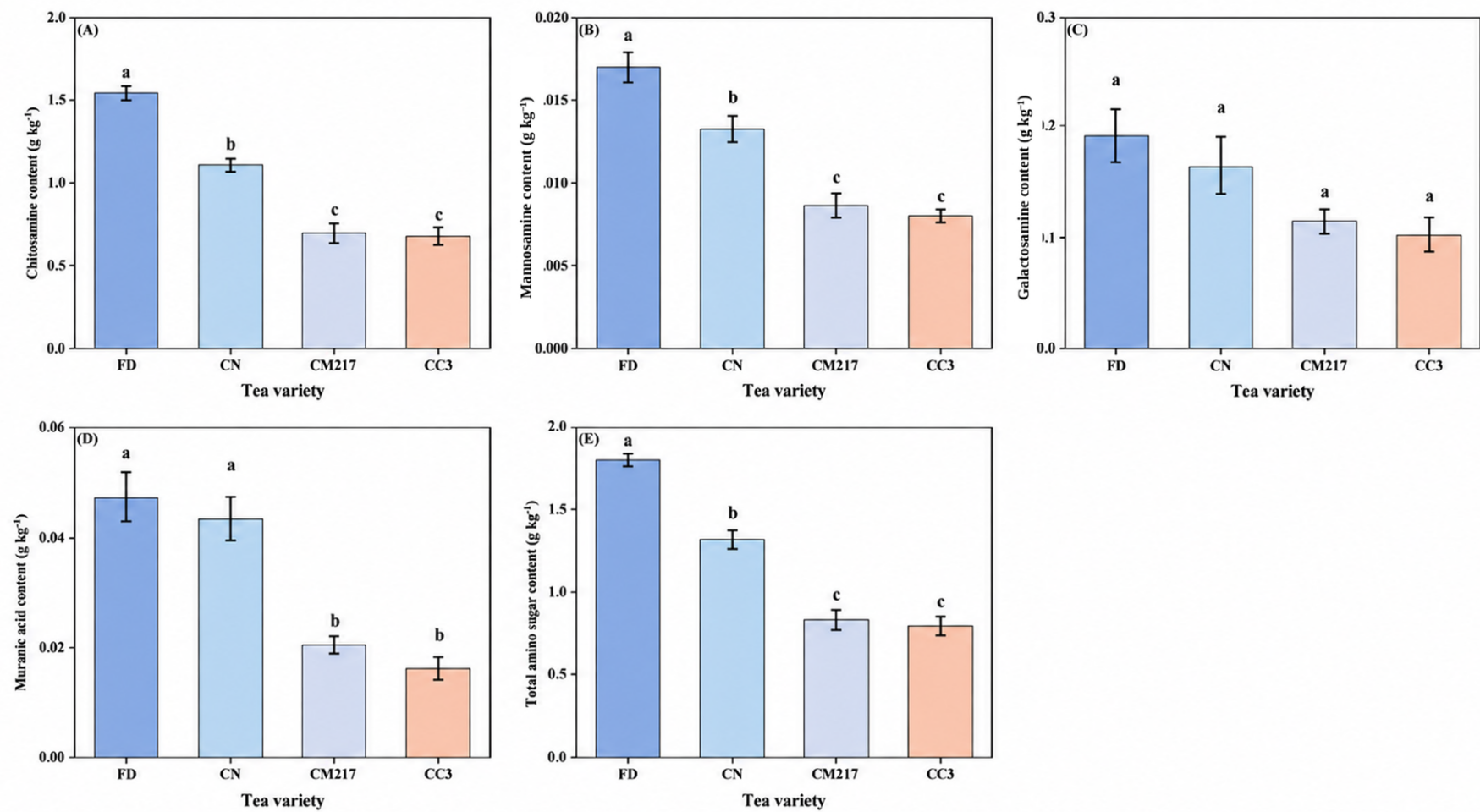


Fig. 2

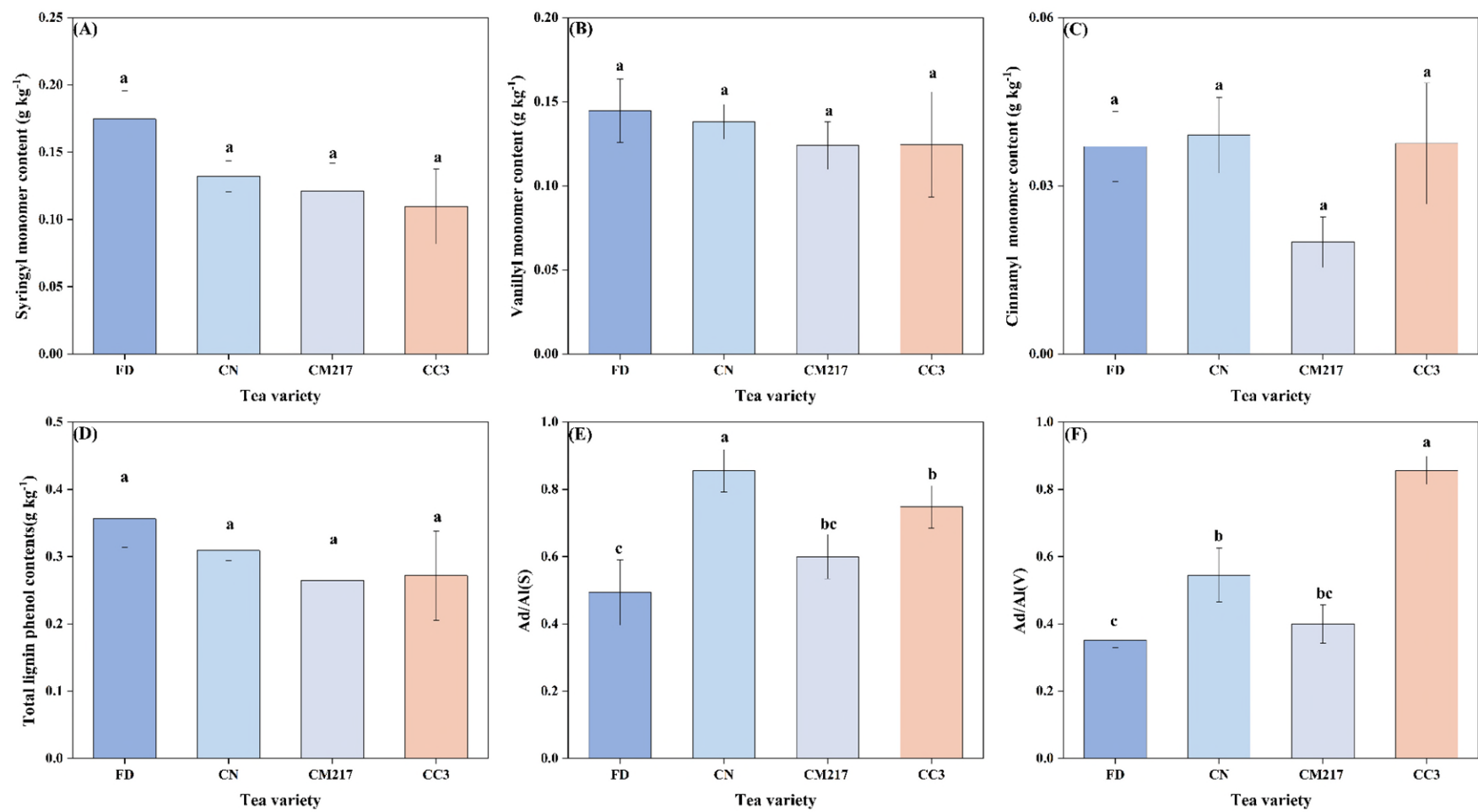


Fig. 3

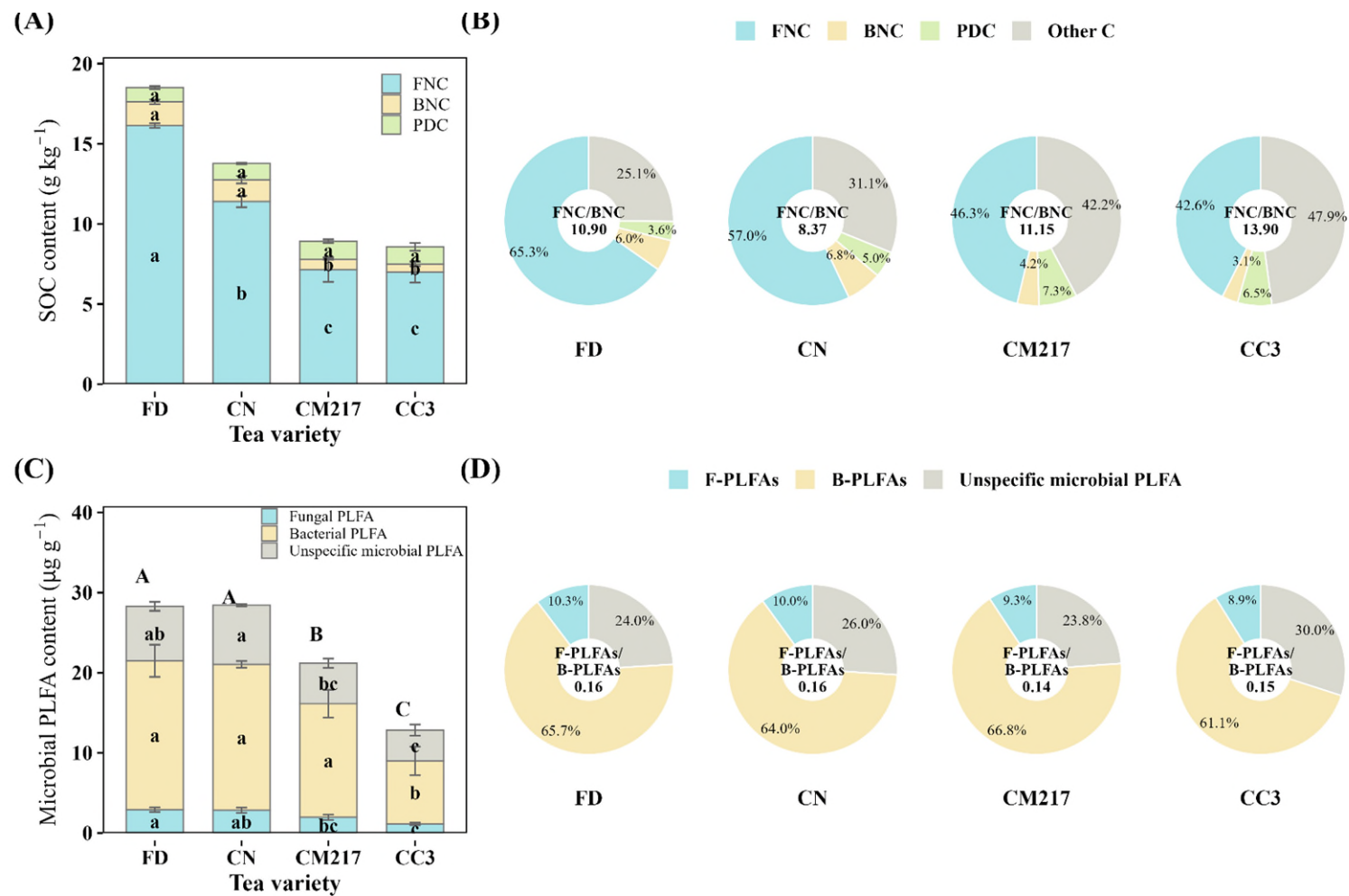


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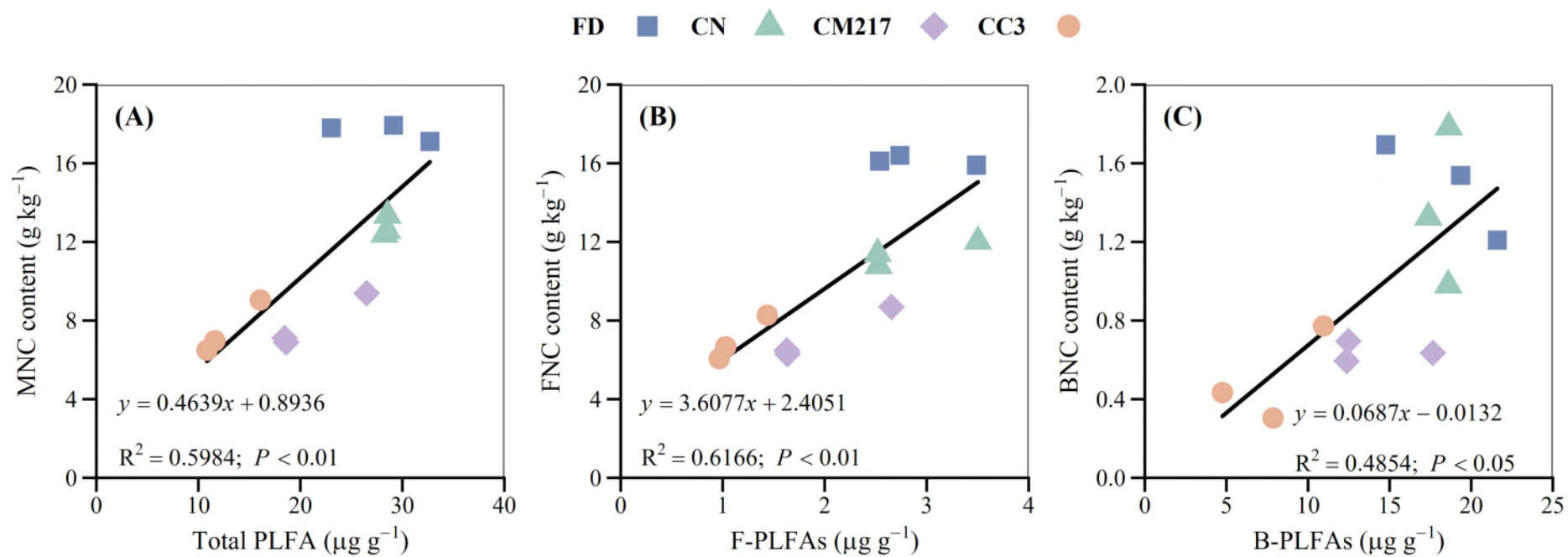


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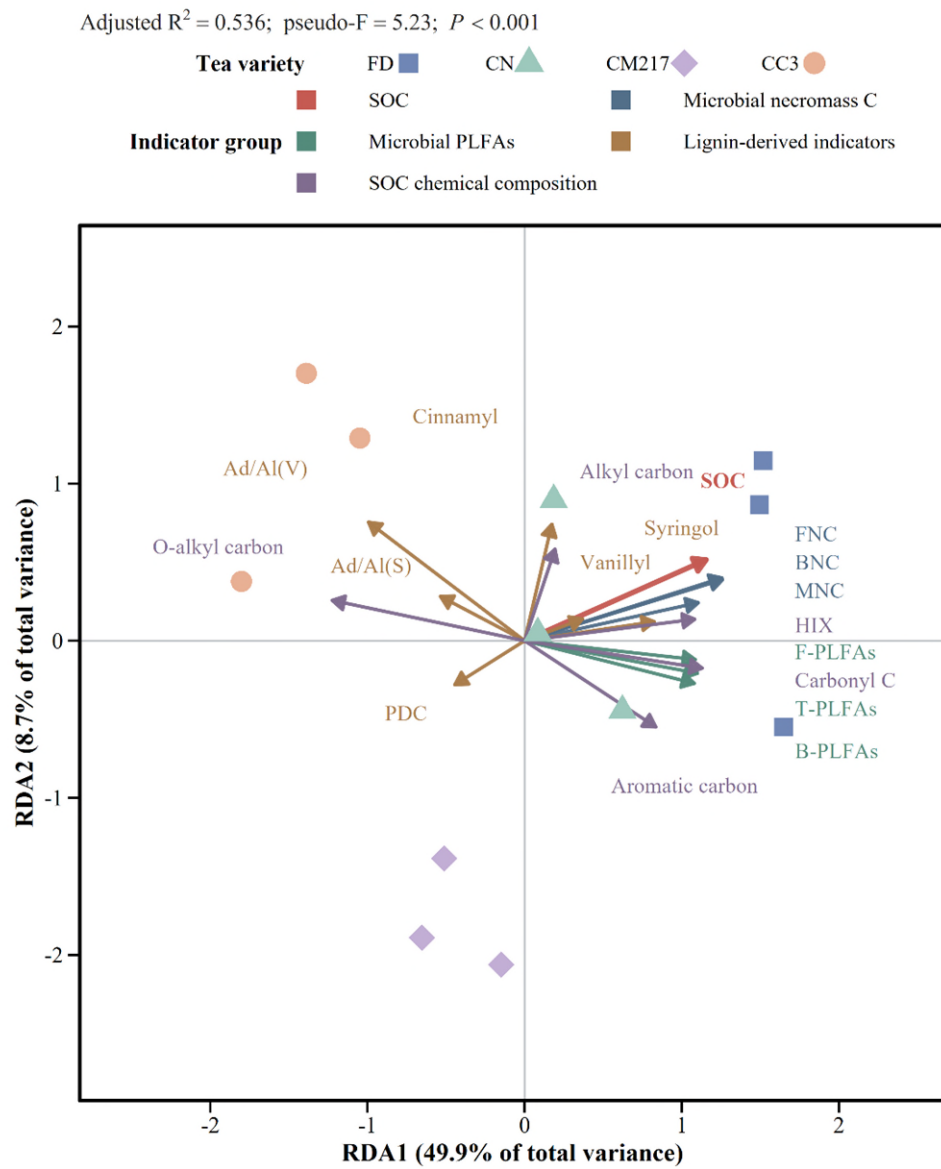


Fig. 6

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