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1 Responses of tropical forest soil organic matter pools to shifts in precipitation
2 patterns

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Abstract

Subsoils contain more than half of global soil organic matter (SOM) stocks. Given that sequestration and turnover processes of SOM are slower in the subsoil than in the topsoil, subsoil carbon (C) stocks are likely to be vulnerable to shifts in precipitation patterns. Therefore, we investigated the responses of different sources of tropical forest soil organic C (SOC) pools to a delayed onset and increased intensity of seasonal precipitation in a 10-year rainfall manipulation experiment. While total SOC varied with soil depth, regardless of shifts in rainfall pattern, we observed that changes in precipitation patterns affected the composition of SOC pools. A delayed wet season increased both the content and proportion of the light fraction C in the SOC at the 0–10 cm depth, potentially due to a decrease in the light fraction decomposition. The delayed wet season also led to a higher content of iron (Fe)-bound organic C, due to impacts on free iron (Fe^{3+}) and aluminum oxides. In addition, wetter wet season led to a higher content of lignin phenols in the top- and subsoil (0–70 cm), due to anoxic conditions preventing lignin decomposition. However, this precipitation shift decreased both the content and proportion of fungal necromass in the SOC in the subsoil (50–70 cm), this was attributed to fungal necromass decomposition by microorganisms facilitated by increased N-acquisition enzyme activity. Overall, greater precipitation intensity increased the vulnerability of subsoil C to losses, primarily due to greater microbial decomposition under increased N limitation. Our study demonstrates the subsoil C-cycling processes in shaping SOM stocks to global changes in precipitation patterns.

Keywords: Tropical forest, Rainfall changes, Soil carbon fractions, Microbial necromass, Lignin phenols, mineral bound carbon

1. Introduction

Global climate warming is causing changes in global and regional rainfall patterns, disrupting the natural water cycle processes (Wang et al., 2021c). For example, climate change is expected to delay sub-tropical and tropical rainy seasons (Biasutti and Sobel, 2009; Seth et al., 2013; Song et al., 2018) and increase levels of rainy season precipitation (Zhou et al., 2011), which is likely to impact plant and soil carbon (C) pools (Sun et al., 2020; Ham et al., 2023). Approximately 30% of global soil C pools are stored in tropical regions, particularly in forest ecosystems, where decomposition of soil organic matter (SOM) accounts for 30%–50% of respired C from tropical forests (Nottingham et al., 2020). Given this substantial contribution, there is an urgent need for quantifying SOM storage and stability in tropical forest under ongoing climate change and shifts in rainfall patterns (Xu et al., 2024).

Elevated precipitation levels modify SOM storage and stabilization by altering C inputs and outputs (Wieder et al., 2009; Yu et al., 2020). This increase in precipitation leads to higher litter inputs (Ryan et al., 2010) and root biomass (McCulley et al., 2007), which ultimately increases the SOM content (Cleveland et al., 2006). However, increased plant–soil C inputs do not necessarily result in net C increase, particularly if these inputs are offset by accelerated litter and SOM decomposition (Jiang et al., 2013; Xu et al., 2024). While empirical evidence primarily focuses on living (biomass) OM, which undergoes fast cycling, little is known about dead (necromass) OM (slowly cycling) derived from plant and microorganisms under elevated precipitation levels. Notably, mounting evidence shows that the stability and contribution of plant residues to SOM have been overestimated (Thevenot et al., 2010), and that microbial necromass plays a greater role in slowly cycling SOM pools, potentially leading to SOM accumulation (Khan et al., 2016; Liang et al., 2019).

Microbial necromass accumulation and composition are influenced by the aridity index and precipitation levels (Amelung et al., 1999; Ma et al., 2018; Hu et al., 2023). A meta-analysis showed a negative relationship between microbial necromass accumulation and mean annual precipitation (Chen et al., 2020). Accelerated rates of plant biomass and rhizodeposition decomposition under increased precipitation levels stimulate microbial biomass growth, necromass accumulation, and the formation of stable SOM in tropical forests (Cleveland et al., 2006; Liang et al., 2017; Zhou et al., 2022). Conversely, rainfall intensification induces nutrient leaching from soil and subsequently nutrient limitation, accelerating microbial mineralization of plant residues and microbial necromass to meet nutrient demands (Cui et al., 2020; Wang et al., 2021a). Gram-negative bacteria and fungi exhibit greater sensitivity to increased precipitation, likely due to the enhanced soil conditions brought about by anaerobicity under increased precipitation, whereas gram-positive bacteria are less affected (Wagner et al., 2015). This indicates the composition of microbial necromass will be influenced by increased rainfall. Seasonal shifts in global and regional precipitation patterns are a consequence of climate change (Song et al., 2018). A delay in precipitation results in a decrease in organic matter decomposition (decreased β -1,4-glucosidase activity) in subtropical and tropical forests (Zhao et al., 2018), and a reduction in above-ground net primary productivity in sub-Mediterranean grasslands (Chelli et al., 2016). A delayed wet season has been shown to increase both the fungal and bacterial biomass owing to a prolonged life cycle of microorganisms (Zhou et al., 2022). Other studies have suggested that delayed rainy seasons influence soil fungal communities more than bacterial communities in subtropical forests (He et al., 2017; Zhao et al., 2017). Such shifts may alter the contributions of fungal necromass-C to SOM, which typically tend to be more substantial than those of bacterial contributions in forest soils (Liang et al.,

2019; Wang et al., 2021a), particularly in tropical forests (Chen et al., 2020). Given the potential impacts of increased precipitation on plant litter and the microbial community structure and necromass, it is important to understand the responses and implications of SOM pool dynamics to changes in C inputs in the context of climate change.

Subsoils store more than half of the global SOM stocks (Li et al., 2020; Button et al., 2022). Increasingly, results from previous studies indicate that deep SOM, despite its relative isolation, is more sensitive than topsoil to precipitation changes (Deng et al., 2023; Liu et al., 2023). The intensification of precipitation under climate change results in higher levels of water infiltration into soils and leaching of dissolved organic carbon (DOC) into deep soil horizons. The vertical transport of plant-derived dissolved organic inputs can diminish SOM storage and stability via priming effects (Hoang et al., 2017; Bailey et al., 2019). The single and sequential leaching of DOC can also result in increased retention among deeper soil horizons due to stronger sorptive capacities (Si et al., 2018). Alternatively, the stability of SOM in the subsoil is also controlled by iron–aluminum oxides and cations (Chen et al., 2021a). The timing and intensity of rainfall are key drivers of soil redox dynamics (Liptzin et al., 2010), an increase in moisture availability can stimulate iron (Fe) reduction and the re-precipitation of amorphous Fe oxides (Nordt and Driese, 2009), and increase mineral protection of SOM (Du et al., 2020; Yu and Kuzyakov, 2021; Angst et al., 2023). However, Mok et al. (2019) found that Fe oxide reduction could be suppressed under heavy rainfall owing to a reduction in the abundance of Fe-reducing bacteria. Uncertainty regarding subsoil C dynamics hampers accurate estimates of responses of the C cycle to global and regional changes in precipitation patterns (Liu et al., 2023).

We report responses of soil C pools to manipulated shifts in the timing and intensity of seasonal precipitation in a tropical forest, from where previous studies have

shown that increases and delays in seasonal precipitation leads to greater plant C inputs (Yu et al., 2019; Zhou et al., 2022). Specifically, we tested the following hypothesis: (1) Delayed wet season increases Fe reduction and the content of Fe oxides and Fe-bound carbon; (2) Delayed wet season decreases fungal necromass and its proportion in SOM due to delayed wet season influence soil fungal communities more than bacterial communities in subtropical forests (He et al., 2017; Zhao et al., 2017); (3) Wetter wet seasons enhance anoxic conditions, especially in subsoil, thereby preventing lignin oxidation and thus increasing plant residues in SOM; (4) Wetter wet seasons reduce fungal biomass due to O₂ limitation (Unger et al., 2009; Hawkes et al., 2011), thereby decreasing fungal necromass formation.

2. Materials and methods

2.1. Study location

The study was conducted in a secondary tropical forest at the Xiaoliang Tropical Coastal Ecosystem Research Station of the Chinese Academy of Sciences in southern China (21°27'N, 110°54'E). Mean annual temperature and precipitation of the prevailing tropical monsoon climate are 23 °C and 1400–1700 mm, respectively, with 70% of the annual precipitation occurring during the rainy season (seasonal precipitation; April to September) (Sun et al., 2020). A previous study has provided detailed descriptions of the dominant trees and shrubs species of the secondary tropical forest (Yu et al., 2019).

2.2. Experimental design

The precipitation manipulation experiment (2013–2023) was based on a completely randomized block design, comprising four replicates of three treatments (ambient precipitation as a control, delayed wet season, and wetter wet season) applied to 12 × 12 m plots that were enclosed by PVC boards to minimize lateral water movements and

separated by a minimum distance of at least 2 m.

Rain exclusion shelters (2.5 m high), comprising 95% light transparency polyethylene sheets on stainless steel framework supports, were used to cover 60% of the plots under delayed wet season, from April to May, to minimize shading effects (da Costa et al., 2010). From June to March, the sheets were drawn back to allow precipitation throughfall (Figure S1). We simulated a delay in the end of the rainy season by installing nine uniformly distributed rotating sprayers (1 m high) that provided the amount of rainfall excluded during the April–May period, distributing it across weekly intervals throughout period October–November (Yu et al., 2022).

We simulated a wetter wet season by irrigating plots with 50 mm of groundwater for 4h at weekly intervals in July and August; this additional 400 mm of water approximated to 25% of mean annual precipitation. A previous study reported that this experimental delay in the onset of the rainy season increased soil moisture during the dry season, while the increase in seasonal precipitation increased soil moisture during the rainy season (Yu et al., 2022).

2.3. Soil and litterfall sampling

After removing surface litter from each experimental plot, we collected five soil cores using an auger (5 cm diameter) at depths of 0–10, 10–20, 20–30, 30–50, and 50–70 cm in March 2023. The soil samples were homogenized to form single composite samples per depth and then immediately transported to the lab prior. They were then passed through a 2 mm mesh under field-moist conditions.

Living roots (≤ 2 mm in diameter) were extracted from the samples and washed with water to remove attached soil particles. They were then dried at 60 °C for 48 h to determine fine root biomass. Then, the soil subsamples were either air-dried, stored at 4 °C, or freeze-dried, prior to chemical, enzyme, and microbial necromass and

community analyses, respectively.

Single litter traps (1×1 m) were placed 1.2 m above the soil surface at random locations within each plot from August 2022 to August 2023. The litterfall that was collected in these traps was dried at 65 °C until reaching constant weight.

2.4. Analysis of soil physicochemical properties

Soil moisture was determined gravimetrically at 105 °C for 24 h and SOM content was determined using the potassium dichromate oxidation external heating method (Sun et al., 2020). The dithionite-citrate-bicarbonate method was used to extract free Fe and Al oxides (Mehra and Jackson, 1958). We used 0.2 M NH_4 -oxalate and 0.1 M $\text{Na}_2\text{P}_2\text{O}_7$ solutions to extract amorphous Fe/Al oxides (McKeague et al., 1971) and organic-bound Fe (Fep) and Al (Alp) (Loveland and Digby, 1984), respectively. The concentrations of cations and metals in the extracts were determined using inductively coupled plasma optical emission spectroscopy (Optima8300; PerkinElmer, Waltham, MA, USA).

Light and heavy soil fractions (<1.8 and >1.8 g cm^{-3} , respectively) were determined using the density fractionation method (Ye et al., 2018), where 10 g of air-dried soil and 60 mL of NaI solution were added to a 100 mL polycarbonate centrifuge tube at a density of 1.8 g cm^{-3} . Then, the mixture was shaken at 300 rpm for 2 h, then centrifuged at 3000 g for 10 min, and allowed to rest for 24 h before the supernatant was removed by pipetting. This procedure was repeated three times per sample, to ensure complete recovery of the light fraction. The light and heavy fractions were washed with deionized water to remove residual NaI before being dried in an oven at 75 °C and ground into powder for organic C analysis using a C/N analyzer. We extracted the heavy fraction using a 0.5 M solution of Na_2SO_4 prior to determination of Calcium (Ca)-bound organic C using a TOC analyzer (Ye et al., 2018). We extracted the Fe-bound organic C using

Na₂SO₄, following the method described by (Lalonde et al., 2012). After which, the residual soil (0.25 g dry soil) was extracted three times using 15 mL of CBD buffer solution (0.27 M trisodium citrate, 0.11 M sodium bicarbonate, and 0.25 g of sodium dithionite) in a water bath at 80 °C for 15 min. After cooling, the mixture was centrifuged at 3000 × *g* for 10 min and the CBD-treated residues were rinsed three times with 1 M NaCl solution. The residual soil samples were also extracted using a 15 mL buffer solution (1.6 M NaCl and 0.11 M sodium bicarbonate) as a control. Organic C content of the extracted residues was determined after drying (75 °C), using an elemental analyzer. We calculated Fe-bound organic C content as the difference between the treatment and control groups.

2.5. Analysis of metrics of soil microbe mass and activity

We analyzed the soil content of phospholipid fatty acids (PLFA) as an indicator of microbial biomass (Bossio and Scow, 1998), following extraction from 8 g of freeze-dried soil using a 23 mL buffer solution of chloroform, methanol, and phosphate (1:2:0.8 v/v/v). We calculated PLFA content according to the 19:0 internal standard concentrations and biomass of the individual fatty acids as nmol lipid g⁻¹ of dry soil. We calculated biomass of bacteria as the sum of i15:0, a15:0, 15:0, i16:0, a16:0, 16:1ω7, i17:0, a17:0, 17:0, cy17:0, 18:1ω7c, and cy19:0ω8c, while biomass of fungi was calculated as the 18:2ω9c. In addition, the biomass of arbuscular mycorrhizal fungi (AMF) was represented by 16:1ω5c, and the biomass of actinobacteria was calculated as the sum of 16:0 10-methyl, 17:0 10-methyl, and 18:0 10-methyl.

Glucosamine (GluN), galactosamine (GalN), and muramic acid (MurN) are amino sugar biomarkers for microbial necromass (Shao et al., 2021). Thus, soil content of amino sugars was determined using the method described by Zhang and Amelung (1996), where 0.5 g of freeze-dried soil that had been ground and passed through a 0.15

mm sieve was hydrolyzed with 10 mL of 6 M HCl at 105 °C for 8 h in a 25 mL hydrolysis tube. The hydrolysate was transferred to a 50 mL centrifuge tube and centrifuged at 3000 rpm for 5 min, before the supernatant was transferred to a clean centrifuge tube and dried using nitrogen gas. Following this, the dried residues were dissolved in 10 mL of deionized water and purified through a series of steps: NaOH neutralization, HCl precipitation of metal ions, and followed by desalination with anhydrous methanol. The purified amino sugars were reacted with 0.5 mL derivative reagent in a derivative bottle, heated in a 75 °C water bath for 35 min, and then cooled to room temperature. After which, 1 mL acetic anhydride was added and the mixture was heated again in a 75 °C water bath for 35 min. Following which, it was cooled to room temperature once more and 1.5 mL dichloromethane was added to extract the amino sugar derivatives. Then, 1 mL of 1 M HCl was added to remove the upper inorganic phase from the derivatives using a pipette, while the organic phase containing amino sugars was separated by washing 3 times with 1 mL of deionized water. The extracted amino sugars were then quantified ($\mu\text{g g}^{-1}$) using high-performance liquid chromatography (Agilent 1100, American), and soil necromass C content ($\mu\text{g/g}$) for microbes (MNC), fungi (FNC), and bacteria (BNC) was calculated using conversion factors of 9 and 45 (Engelking et al., 2007). The calculations are as follows:

$$\text{MNC (F-GluN)} = \text{total GluN} - [(2 \times \text{MurN}) \times (179.2/251.2)]$$

$$\text{FNC} = \text{F-GluN} \times 9$$

$$\text{BNC} = \text{MurN} \times 45$$

We analyzed the activity of a range of extracellular soil enzymes ($\mu\text{mol g}^{-1}$ dry soil h^{-1}) as indicators of soil microbe activity. β -glucosidase activity (BG) was determined using 0.025-M p-nitrophenyl- β -D-glucopyranoside as substrate (Eivazi and Tabatabai, 1988). Activities of N-acetylglucosaminidase (NAG) and leucine aminopeptidase (LAP) were

determined using an enzyme kit (Comin Biotechnology Co., Ltd, Suzhou, China) according to the manufacturer's instructions, with 4-nitrophenyl-N-acetyl- β -D-glucosaminide and L-leucine-p-nitroanilide as substrates, respectively (Wang et al., 2022). Acid phosphatase activity (ACP) was assayed following the method described by Tabatabai and Bremner (1969), where 1 g of fresh soil was mixed with 0.2 mL toluene, 4 mL modified universal buffer (MUB), and 1 mL p-nitrophenyl phosphate solution (0.224 M) prior to incubation at 37 °C for 1 h. The amount of p-nitrophenol released by phosphatase activity was determined photometrically at 400 nm. Phenol oxidase and peroxidase activities were measured spectrophotometrically using L-3,4-dihydroxyphenylalanine (DOPA) as the substrate (Saiya-Cork et al., 2002). For phenol oxidase, 50 μ L of 10 mM DOPA was added to each sample well. Peroxidase assays received 50 μ L of 10 mM DOPA plus 10 μ L of 0.3% H₂O₂. For phenol oxidase, blank wells contained 50 μ L of acetate buffer. For peroxidase, blank wells also contained 50 μ L of acetate buffer plus 10 μ L of 0.3% H₂O₂. There were 3 replicate sample wells for each assay and 3 replicate wells for blanks. The microplates are incubated in the dark at 20 °C for 21 h. The enzyme activities were measured by the Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific) at a wavelength of 450 nm and expressed as $\mu\text{mol g}^{-1} \text{ dry soil h}^{-1}$.

2.6. Analysis of indicators of plant-derived soil organic carbon

We analyzed soil lignin phenol content as indicators of plant derived SOC using the copper oxide (CuO) oxidation method (Otto et al., 2005), where 1 g of freeze-dried soil was pretreated by solvent extraction and alkaline hydrolysis to remove extractable and hydrolyzable lipids, respectively, and then mixed with 1 g of CuO, 100 mg of Fe (NH₄)₂(SO₄)₂·6H₂O and 20 ml of 2-M N₂-purged NaOH solution in Teflon-lined bombs heated at 170 °C for 2.5 h under N₂. The lignin oxidation products were spiked with a

surrogate standard (ethyl vanillin), with pH adjusted to 1 using 6-M HCl under dark conditions for 1 h. After centrifugation, the lignin oxidation products were extracted three times using ethyl acetate from the clear supernatant, spiked with a quantitative standard (trans-cinnamic acid), and concentrated with N₂, prior to derivatization with N, O, -bis-(trimethylsilyl) trifluoroacetamide and pyridine at 70 °C for 3 h. The derivatized lignin phenol compounds were identified using a gas chromatography mass spectrometer (GC–MS) QP 2010 PLUS, Shimadzu) and comprised vanillyls (V; vanillin, acetovanillone, and vanillic acid), syringyls (S; syringaldehyde, acetosyringone, and syringic acid), and cinnamyls (C; p-coumaric acid and ferulic acid). Total lignin phenol content was determined as the sum of V, S, and C (Chen et al., 2021b).

2.7. Statistical analysis

Statistical analyses were performed using SPSS v. 17.0 (SPSS Inc., Chicago, IL). Shapiro–Wilk and Levene tests were used to test data normality and homogeneity, respectively, before analysis. We used generalized linear mixed model (GLMM) analysis to estimate effects of shifts in rainfall on SOM, soil C fractions, and metrics of soil microbe abundance and activity across the soil depths. One-way ANOVA (with Tukey tests) was used to test for within-soil depth effects of precipitation changes on C fractions, amino sugars and microbial community and biomass at $p < 0.05$.

3. Results

3.1. Plant biomass, soil organic matter, and chemical properties

Compared to the control plots without precipitation changes, the delayed wet season and the wetter wet season showed no differences in litterfall biomass and total fine root biomass, but delayed wet season increased fine root biomass in the 20–30 cm soil depth ($p < 0.05$; Figure 1a, b). Overall, the total SOM content in the control, the

delayed wet season and the wetter wet season remained stable, but decreased with depth ($p < 0.001$) (Figure 1c, d).

The delayed wet season, wetter wet season and the control exhibited similar contents of total C, total N, C/N, soil water content, and pH across all depths (Figure S2). The delayed wet season decreased amorphous soil content of Fe oxides in the subsoil (20–70 cm) (Figure S3a) and increased the soil content of free Fe and Al oxides in the 0–10 cm soil layer (Figure S3c, d) ($p < 0.05$). A wetter wet season led to a reduced content of amorphous Fe oxides in the subsoil (20–70 cm) (Figure S3a) and a greater content of organic Fe and Al oxides in the 0–10 cm soil depth (Figure S3e, f) ($p < 0.05$).

3.2. Soil C pools

Total necromass C accounted for a large portion of SOM, ranging from an average (across all treatments) of $69 \pm 3.9 \text{ g kg}^{-1}$ at the 0–10 cm soil depth to $40 \pm 4.5 \text{ g kg}^{-1}$ at the 50–70 cm soil depth (Figure 2). FNC in SOC ranging from an average (across all treatments) of $49 \pm 2.8 \text{ g kg}^{-1}$ at the 0–10 cm soil depth to $24 \pm 2.3 \text{ g kg}^{-1}$ at the 50–70 cm soil depth (Figure 2). BNC in SOC ranging from an average (across all treatments) of $20 \pm 1.2 \text{ g kg}^{-1}$ at the 0–10 cm soil depth to $16 \pm 2.3 \text{ g kg}^{-1}$ at the 50–70 cm soil depth (Figure 2). There was a greater proportion of FNC in SOC than BNC (Figure 2c–f). The wetter wet season led to a decrease in the content and proportion of FNC in SOC at 50–70 cm soil depth (Figure 2e, f), and led to an increase in the content and proportion of total lignin phenols in SOC at 0–10, 20–30 and 50–70 cm soil depths (Figure 2g, h). Compared to the control, a delayed wet season increased the content of total lignin phenols at the 0–20 cm soil depths (Figure 2g). However, there were no differences in the proportions of total lignin phenols in SOM throughout the soil depths (Figure 2h). A wetter wet season increased the content of V- and S-type phenols at soil depths >20 cm, along with an increase in their proportions in SOC at 20–30, and 50–70 cm soil

depths (Figure S4a–d). The wetter wet season also increased the content and proportion of C-type lignin phenols in SOC at the 20–30 cm soil depth (Figure S4e, f) ($p < 0.05$). Furthermore, the delayed wet season increased the content and proportion of C-type phenols in SOC at the 10–20 cm soil depth ($p < 0.05$; Figure S4e, f).

There were discernable effects of shifts in precipitation patterns on physicochemical sources of soil C in the upper 0–10 cm depth (Figure 3). The delayed wet season increased the content and proportion of the light fraction C and Fe-bound C in the SOC, and decreased the content and proportion of Ca-bound C in the SOC (Figure 3a, b, e–h). Similarly, the wetter wet season increased the content and proportion of light fraction C in the SOC (Figure 3a, b) ($p < 0.05$).

3.3. Soil microbial biomass and enzyme activities

The activity of extracellular soil enzymes (Figure 4) varied with soil depth, regardless of shifts in precipitation patterns. There were no treatment effects on activity of BG, ACP or peroxidase; however, the activity of LAP in the 0–10 cm soil depth increased under more intense season precipitation, along with activity of NAG in the 30–50 and 50–70 cm soil depths ($p < 0.05$; Figure 4). Wetter wet season led to a decrease in soil phenol oxidase activity at the 10–20, 20–30, 30–50 and 50–70 cm depths ($p < 0.05$; Figure 4).

The delayed wet season and the control exhibited similar contents of total PLFA, fungal PLFA, bacterial PLFA, actinobacterial PLFA, AMF PLFA biomass, and fungal:bacterial ratio across all depths (Figure S5). However, the wetter wet season led to a greater soil content of total PLFAs at the 10–20 cm depth, AMF PLFAs at the 0–10 cm depth, and actinobacterial PLFAs at the 10–20, 20–30, 30–50 and 50–70 cm depth ($p < 0.05$; Figure S5).

4. Discussion

Over the 10-year experimental period, the total SOC content remained stable in both the top- and subsoil of the tropical forest ecosystem, showing no measurable changes in response to shifts in precipitation patterns. However, we observed that the delay in the onset of the rainy season and increased intensity in rain season precipitation led to changes in SOC composition, with a divergence in the contributions of plant- and microbe-derived C to SOC, both in the top- and subsoil.

4.1. Delayed wet season increased Fe-bound C in topsoil SOC

The delayed wet season led to increases in the light fraction C pools in the topsoil (Figure 3), likely a delay in precipitation results in decrease in organic matter decomposition in subtropical and tropical forests (Zhao et al., 2018). We observed that the delayed wet season resulted in an increase in the association of the SOC with mineral components and Fe-bound organic C, along with increases in soil free Fe/Al oxides (Figure S3). This phenomenon may be attributed to the delayed rain season allowing for a period with more oxygen levels in soil, thereby favoring the formation of the more insoluble Fe^{3+} under drier conditions. This, in turn, promotes the precipitation and formation of hematites (Fe_2O_3) and organic Fe complexes (Hsu and Wang, 1980; Hall and Silver, 2013; Song et al., 2022). These results were consistent with the first hypothesis. Therefore, our study aligns with previous studies that show the timing of rainfall is a major driver of fluctuations in soil redox environment, due to impacts on Fe reduction and the content of Fe/Al oxides (Liptzin et al., 2010; Angst et al., 2023).

In line with previous research from subtropical forests (Zhao et al., 2017), we observed minor effects of the delayed wet season on microbial biomass, enzyme activities, and microbial necromass (Figure 2, 4, S4), indicating a limited sensitivity of tropical forest soil microorganisms to timing of seasonal precipitation (Yu et al., 2022).

This absence of any response in terms of the microbial biomass may explain the lack of impact observed on necromass and enzyme activities (Figure 2, 4).

We observed that neither fine root biomass nor annual litterfall were affected by the delayed wet season in our tropical forest study area (Figure 1), which suggests subtropical-tropical forests have greater resilience. This lack of impact is likely due to the absence of water stress (Fig. S2) and the associated reduction in plant growth (Wang et al., 2018) that occurs when soil moisture is limited during the growing season (Zeppel et al., 2014). Notably, our observations from this tropical forest contrast with those for other ecosystems. For example, Gaul et al. (2008) found that delayed onset of the rainy season in boreal spruce forests increased fine root turnover and the associated input of root-derived C to the soil organic matter pool. Chelli et al. (2016) showed that a dry spring leads to lower above-ground net primary productivity in sub-Mediterranean grasslands, despite increases in summer rainfall.

Contrary to our second hypothesis, we did not find any evidence supporting a decrease in the content and proportion of soil organic carbon (SOC) derived from fungal necromass in response to a shift in the onset of seasonal precipitation. However, our study confirms the predominance of soil organic carbon (SOC) accumulations in the topsoil of subtropical forests, primarily driven by inputs of Fe-bound and plant-derived carbon compounds, including lignin phenols, light fraction, and particulate organic matter (Jia et al., 2021).

4.2. More intense seasonal precipitation decreases fungal necromass in subsoil SOC

The increase in soil content of lignin phenols under more intense seasonal precipitation (Figure 2) was likely not in response to greater plant aboveground and belowground C pools (Yue et al., 2017; Rocci et al., 2023). This conclusion is supported by the lack of effects we observed on the biomass of annual litterfall and fine roots, as

well as reported impacts on rates of litter decomposition (Yu et al., 2019). Instead, the increase can be attributed to the resulting anoxic conditions that prevent lignin oxidation (Chen et al., 2021b), as indicated by the increases in subsoil lignin phenol content and associated lower redox conditions. Furthermore, we observed that the content and proportion of vanillyls in SOC were higher than syringyls and cinnamyls under more intense precipitation, reflecting greater resistance to degradation of vanillyls, particularly under anoxic conditions (Thevenot et al., 2010). These results were consistent with the third hypothesis.

As reported in another study (Chen et al., 2020), the high level of contribution of MNC to SOC in our tropical forests study site under ambient precipitation conditions (72% in 0–10 cm depth, Figure 2) was greater than that of temperate forests (30%, Liang et al., 2019), arable and grassland soils (48%; Khan et al., 2016), likely due to the faster rates of litter decomposition and microbial turnover in the tropics (Cotrufo et al., 2015; Cui et al., 2020; Nottingham et al., 2022). We also observed the dominance of FNC over BNC in SOC (Figure 2), due to the greater tolerance of fungi to acidic soil conditions (Rousk et al., 2009). However, increased precipitation intensity led to a decrease in subsoil FNC content due to increased decomposition, as evidenced by the concurrent increases in actinobacterial PLFAs (Figure S4) and N-acquiring enzyme activity (Figure 4), as N-acetylglucosaminidase degrades chitin derived from fungi and peptidoglycan derived from bacteria (Sinsabaugh et al., 2008). Microorganisms may accelerate N-rich microbial necromass turnover to meet N demands for accelerated synthesis of enzymes required for mineralization (Cui et al., 2020).

Decomposition of necromass by priming is a key ecosystem response mechanism to imbalances in C:N stoichiometry (Sokol et al., 2022). For example, chitin, which is the principal component of fungal cells, undergoes rapid mineralization in tropical soils,

due to its low C:N ratios (~10) (Russell, 2014; Tian et al., 2019). Given bacterial necromass is dominant in heavy soil fractions and fungal necromass is dominant in free and occluded light fractions (Griepentrog et al., 2013), fungal necromass is more accessible to microbial action than bacterial necromass, owing to mineral protection. However, increased intensity of precipitation decreased the soil content of amorphous Fe oxides (Figure S3) and associated mineral protection for SOC, this indicates that higher annual rainfall may lead to more rapid turnover of SOM and reduce the accumulation of fungal necromass in subsoils of tropical forests. These results were inconsistent with our forth hypothesis, because fungal biomass was not changed by wetter wet seasons (Fig. S5). Nevertheless, this finding is somewhat unexpected, as a previous meta-analysis has shown contrasting sensitivities of soil C cycles to directional shifts in precipitation in arid (increases) and humid (decreases) ecosystems (Wang et al., 2021b).

5. Conclusions

Quantification of sources and composition of tropical forest SOC in response to long-term changes in rainfall patterns allows for the conceptual estimation of soil C-cycling processes (Figure 5). Our study found that shifts in precipitation over a 10-year period affected the chemical composition of SOC derived from plants and microbes. The decline in subsoil FNC and SOC stocks (50–70 cm) observed with increased rainfall provides clear evidence for a positive soil C-climate feedback loop. Thus, our study further strengthened our understanding of subsoil C-cycling processes to global precipitation changes in forest soils.

CRedit authorship contribution statement

Feng Sun: Writing – original draft, Writing – review & editing, Visualization, Formal analysis, Data curation, Conceptualization, Funding acquisition. **Linan Fan:**

Methodology, Investigation. **Guangyan Deng:** Investigation. **Yakov Kuzyakov:** Writing – review & editing, Funding acquisition. **Jinchuang Wang:** Writing – review & editing. **Yingwen Li:** Review & editing, Project administration. **Faming Wang:** Writing – review & editing, Project administration. **Zhian Li:** Project administration. **Akash Tariq:** Writing – review & editing. **Jordi Sardans:** Writing – review & editing, Funding acquisition. **Josep Penuelas:** Writing – review & editing, Funding acquisition. **Mei Wang:** Writing – review & editing. **Changlian Peng:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Figure captions

Figure 1 Linear mixed model estimates and ANOVA of effects of shifts in precipitation patterns on biomass of annual litterfall and fine root, and soil organic carbon (SOC) content across and within soil depths, respectively. Green, orange, and blue symbols/lines are control, delay in onset of rainy season, and increased intensity of rainy season precipitation, respectively. Error bars represent standard errors of the means ($n = 4$); different letters indicate significant differences at $p < 0.05$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

Figure 2 Linear mixed model estimates and ANOVA of effects of shifts in precipitation patterns on sources and relative proportion in soil organic carbon (SOC) of microbe and plant C across and within soil depths, respectively. Green, orange, and blue symbols/lines are control, delay in onset of rainy season, and increased intensity of rainy season precipitation, respectively. Error bars represent standard errors of the means ($n = 4$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

Figure 3 Linear mixed model estimates and ANOVA of effects of shifts in precipitation patterns on sources and relative proportion in soil organic carbon of soil physicochemical C fractions across and within soil depths, respectively. Green, orange, and blue symbols/lines are control, delayed wet season, and wetter wet season, respectively; SOC: soil organic carbon; C: carbon; Fe: iron; Ca: calcium. Error bars represent standard errors of the means ($n = 4$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

Figure 4 Linear mixed effect model estimates and ANOVA of effects of shifts in precipitation patterns on activity of soil extracellular enzymes. NAG: N-acetylglucosaminidase; LAP: leucine aminopeptidase; BG: β -1,4-glucosidase; and, ACP: acid phosphatase. Green, orange, and blue symbols/lines are control, delayed wet season, and wetter wet season, respectively. Error bars represent standard errors of the means ($n = 4$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

Figure 5 Conceptual diagram of impacts of long-term shifts in timing and intensity of rainfall

on sources and composition of tropical forest soil organic matter pools, and regulation of soil C cycling and sequestration. Data for litter decomposition rate are reported by Yu et al. (2019).

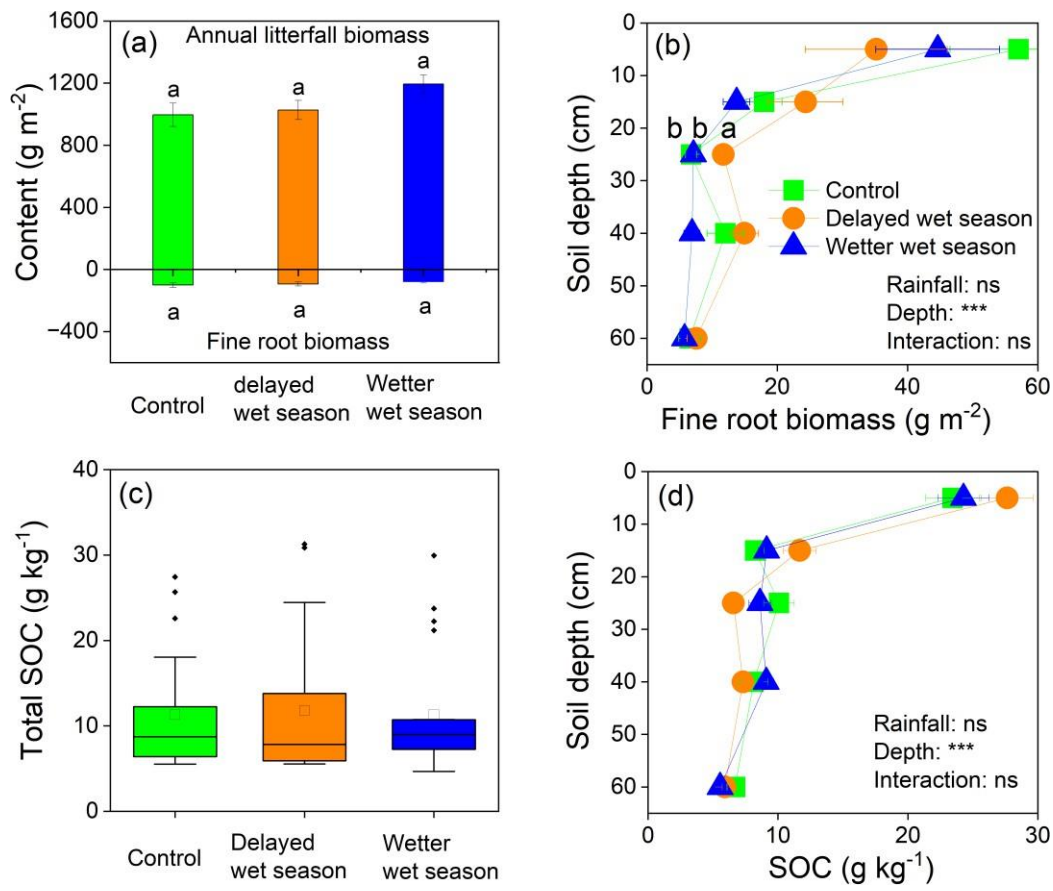
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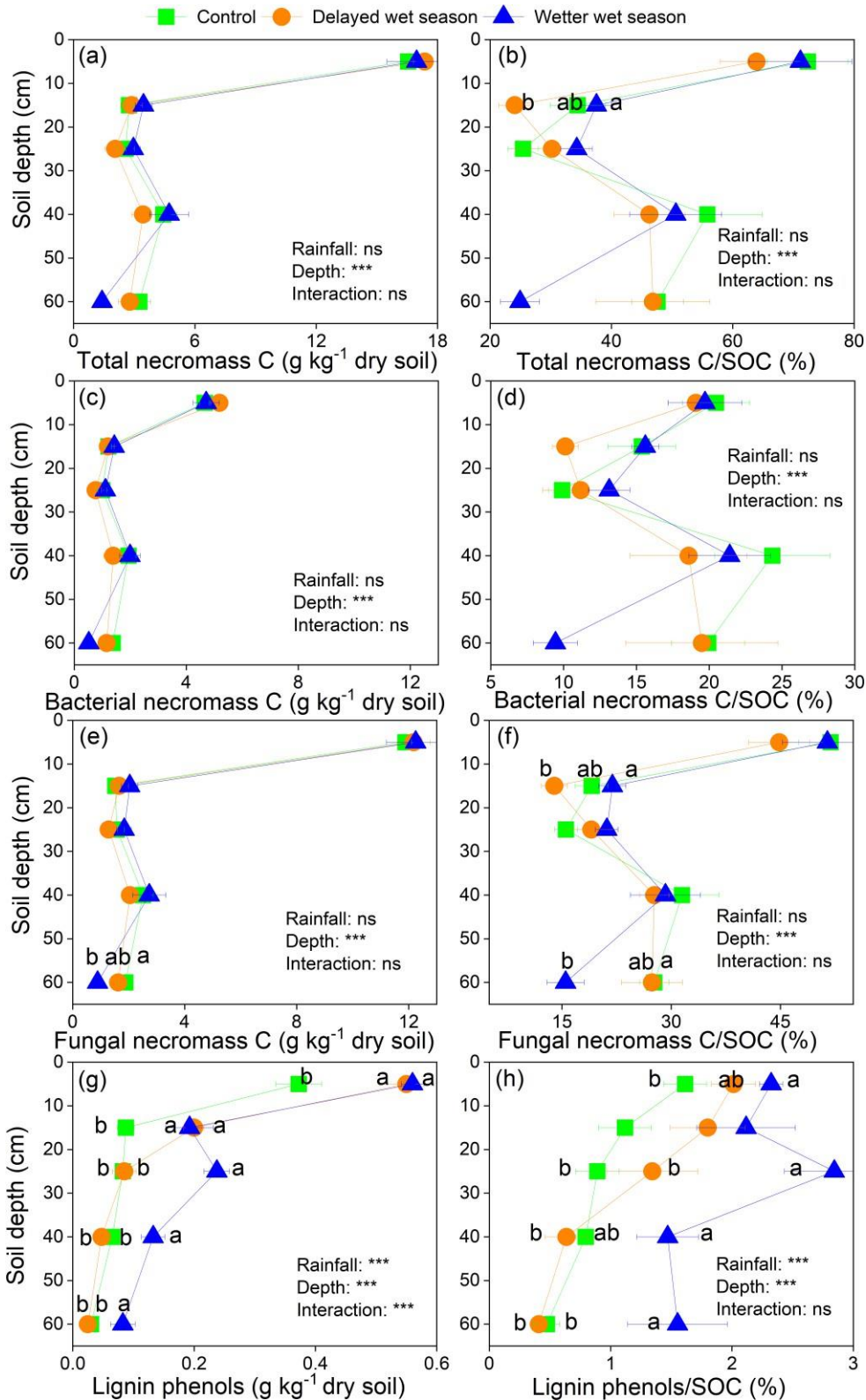
Figure 2

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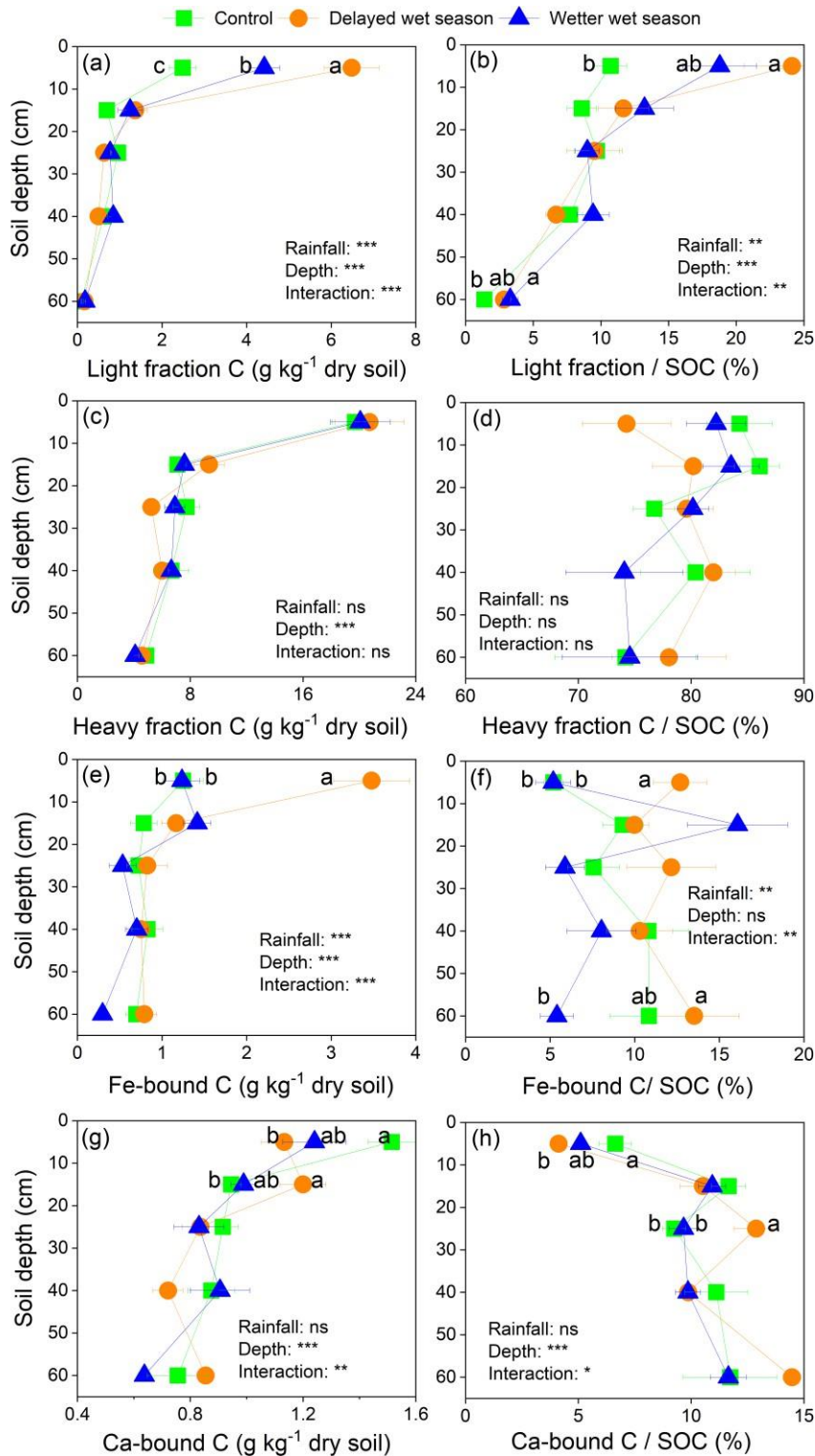
Figure 3

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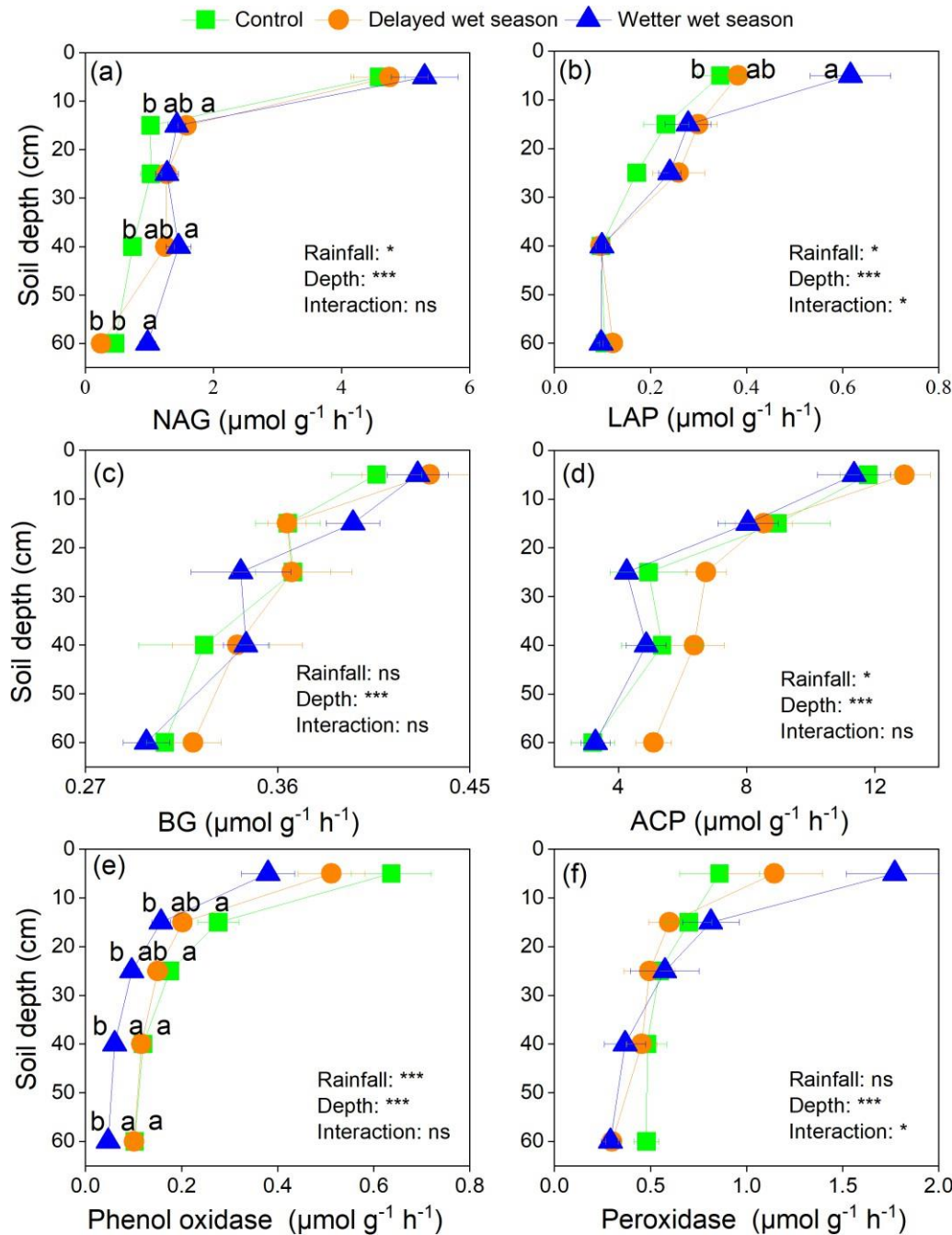
Figure 4

Figure 4 Linear mixed effect model estimates and ANOVA of effects of shifts in precipitation patterns on activity of soil extracellular enzymes. NAG: N-acetylglucosaminidase; LAP: leucine aminopeptidase; BG: β -1,4-glucosidase; and, ACP: acid phosphatase. Green, orange, and blue symbols/lines are control, delayed wet season, and wetter wet season, respectively. Error bars represent standard errors of the means ($n = 4$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

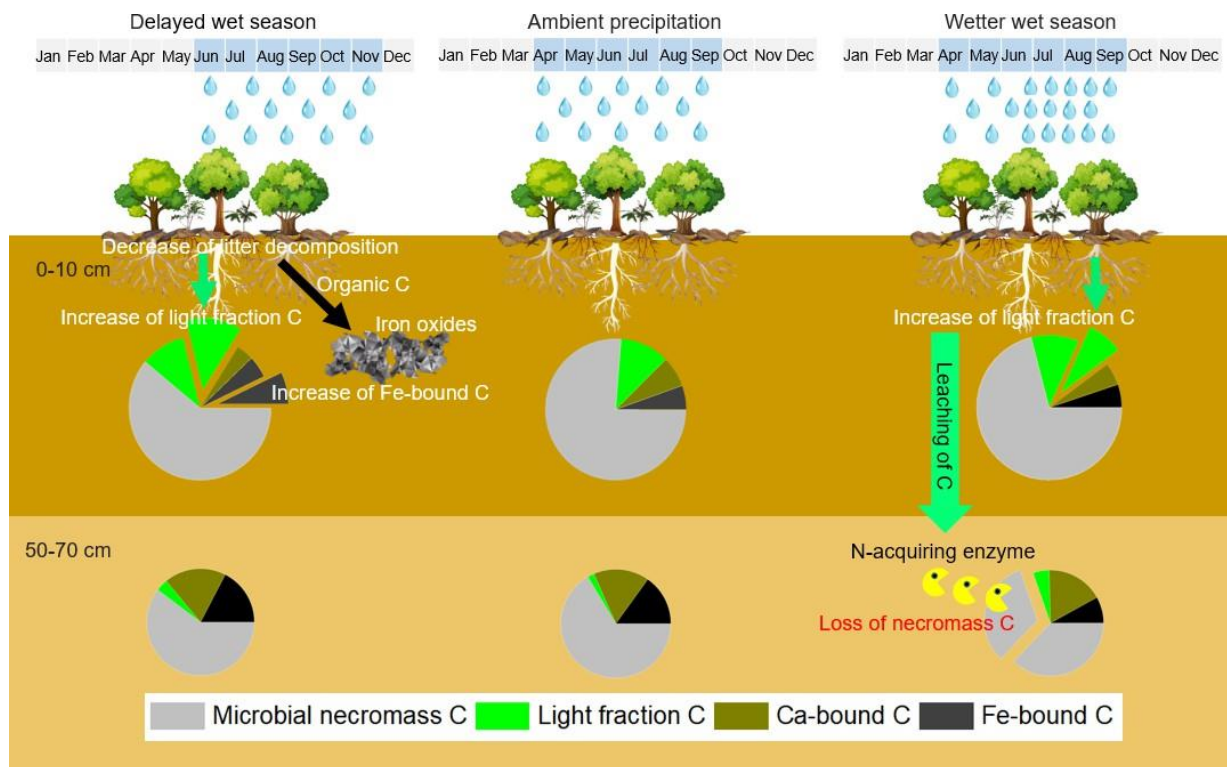
Figure 5

Figure 5 Conceptual diagram of impacts of long-term shifts in timing and intensity of rainfall on sources and composition of tropical forest soil organic matter pools, and regulation of soil C cycling and sequestration. Data for litter decomposition rate are reported by Yu et al. (2019).

Highlights

1. Delayed wet season led to higher content of Fe-bound organic carbon in topsoil.
2. Wetter wet season led to greater content of lignin phenols in soil.
3. Wetter wet season lead to higher subsoil vulnerability by fungal necromass lost.
4. Consider subsoil when assessing the impacts of rainfall changes on soil organic matter pools.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: