University of São Paulo
"Luiz de Queiroz" College of Agriculture

Microbial necromass, carbon and agriculture: combining living and dead microbes to reveal carbon dynamics under agroforestry in the Amazon

Alberto Vinicius Sousa Rocha

Dissertation presented to obtain the degree of Master in Science. Area: Soils and Plant Nutrition

Piracicaba
2024

Alberto Vinicius Sousa Rocha
Bachelor of Agronomy

Microbial necromass, carbon and agriculture: combining living and dead microbes to reveal carbon dynamics under agroforestry in the Amazon

versão revisada de acordo com a resolução CoPGr 6018 de 2011.

Advisor:
Prof. Dr. **FERNANDO DINI ANDREOTE**

Dissertation presented to obtain the degree of Master in Science. Area: Soils and Plant Nutrition

Piracicaba
2024

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Rocha, Alberto Vinicius Sousa

Microbial necromass, carbon and agriculture: combining living and dead microbes to reveal carbon dynamics under agroforestry in the Amazon / Alberto Vinicius Sousa Rocha. - - versão revisada de acordo com a Resolução CoPGr 6018 de 2011. Piracicaba, 2024.

76 p.

Dissertação (Mestrado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz".

1. Sistemas agroflorestais 2. Cultivo de dendê 3. Carbono orgânico do solo 4. Necromassa microbiana 5. Comunidades microbianas do solo I. Título

DEDICATION

To the most important women in my life: Alda Sousa, Maria de Nazaré, and Jéssica Rocha. Thank you so much for being much more than just my mother, grandmother, and sister (respectively) and for supporting me in my decisions, even when you didn't fully understand them at times. I love you all!

ACKNOWLEDGMENT

Agradecer é, por si só, a materialização da nossa indissociabilidade enquanto seres humanos. Ao realizarmos esse ato, evidenciamos a necessidade que temos de nos relacionarmos, dialogarmos e, portanto, de contribuirmos uns com os outros. Contribuir é, por sinal, uma das tarefas mais importantes no universo acadêmico e evidentemente da vida, mas as vezes também difícil de se fazer, não? São sempre muitas variáveis e covariáveis postas à mesa. No entanto, posso dizer que tive o prazer (e porque não dizer o privilégio) de cruzar com pessoas extremamente singulares e que contribuíram, cada uma da sua própria forma e jeito, para o início (2022) e término (2024) dessa caminhada marcada por muitas “aventuras” que convencionamos chamar por mestrado.

Tenho ciência que a forma mais apropriada para escrever essa seção seria em inglês. Contudo, acredito na filosofia que diz que temos a nossa personalidade moldada, em certo grau, pelo idioma que estamos falando. Isso se respalda pelo fato de sermos imersos em uma segunda língua em momentos e condições de socialização diferentes na vida em comparação ao nosso idioma nativo. Dessa forma, sinto que sou uma pessoa mais interessante e as vezes “mais real” em português em função da facilidade que tenho para “manipular” minha língua e, portanto, expressar meus sentimentos. Assim, me apego a liberdade poética que esse tópico me concede e peço a licença para tecer os próximos pontos majoritariamente em português.

Dito isso, gostaria de iniciar agradecendo a minha mãe, Alda Sousa, minha irmã, Jéssica Rocha, e a minha avó, Maria de Nazaré, por todo o amor, carinho e suporte dado ao longo dessa minha curta jornada de vida. Foram muitos altos e baixos - nas mais variadas perspectivas -, mas nos mantemos resilientes e, na medida do possível, seguimos em frente. Sou especialmente grato pela vida da minha mãe que, por um assopro desajeitado da vida, quase nos deixa nesse plano. Porém, conosco está e de modo pulsante comemora essa conquista ao meu lado, que diga-se de passagem é tão coletiva quanto individual. Gratidão profunda ao universo por tê-las comigo e como minha família.

Ao Prof. Fernando Dini Andreote, que, sem saber ou perceber, mudou minha vida ao responder meu e-mail na manhã de uma segunda-feira, 13 de maio de 2019, às 08:24. A partir dessa data, aquele garotinho do interior do Pará ganhou um

supervisor de estágio de iniciação científica (IC), depois um coorientador para seu Trabalho de Conclusão de Curso (TCC) no IFPA e, anos depois, um orientador para seu programa de mestrado na melhor universidade do país. Muito obrigado por ter me dado as condições necessárias para que eu pudesse ir “em frente”, professor. Que o senhor continue sendo esse parceiro para muitas outras gerações!

Aos amigos do grupo de pesquisa por tornarem essa jornada mais leve, divertida e por me ajudarem a me desenvolver: Antonio Marcos (Tony), Alice Alves, Carolinne Rosa, Felipe Martins, Victor Lucas, Elaine Lourente, Thauani Diogo, Izabelly Pacifico e Amanda Oliveira. Da mesma forma, agradeço as queridas e doces amizades que construí e que igualmente contribuíram para o meu desenvolvimento, cada uma da sua própria forma: Maria Gabriella (Gaby PA), Gabriella Sousa (Gabi MA), Higor José, André Espinoza, Heiber Andres, Gabriel Gustavo, Rafael Alves, Jorge Tadeu, Allanna Jerônimo, Lina Marcela e Itallo Cristian.

Um abraço especial à Rafael Santana por todo o carinho, companheirismo e incentivo. És uma pessoa gigante e sou muito feliz por ter cruzado com você nesse caminho! Ao João Xavier e ao Felipe Machado, meus companheiros da subversiva “Casa 2” da Vila de Pós-Graduação da ESALQ/USP. Obrigado por terem me acolhido e tornado meu processo de adaptação e permanência em Piracicaba muito mais alegre e menos solitário. Jamais esquecerei das nossas risadas e conversas.

Aos técnicos do nosso saudoso Laboratório de Microbiologia do Solo: Denise de Lourdes C. Mescolotti e Luis Fernando Baldesin (para nós, a “Dê” e o “Fernandinho”, respectivamente). Muito obrigado pelo suporte dado em absolutamente tudo que se possa imaginar. Vocês são exemplos de servidores públicos e sou muito feliz por ter compartilhado momentos com vocês ao longo desses dois anos e meio. Também estendo meus agradecimentos ao técnico Rodrigo Floriano Pimpinato e ao Prof. Valdemar Tornisielo, ambos do CENA/USP, pelo apoio crucial nas análises de cromatografia líquida.

To Kate Buckeridge, one of the kindest, most respectful, smart, and competent people I have had the privilege of meeting in academia. Thank you so much for agreeing to collaborate on this research and for welcoming me, even without knowing me and Fernando beforehand, at the Luxembourg Institute of Science and Technology (LIST) for my internship. This experience was unique in my life and made me realize that things "are possible" and that there is no "here" and "there". It made me understand, once and for all, that it is possible to “dream". My deepest gratitude to you!

To the researchers and engineers at LIST for their respect, attention, and support during my stay, especially: Michaël Eickermann, Arturo Torres, Matteo Ripamonti, Julie Soulier, Denis Pittois, Céline Lett, Marine Pallez, Ivone Trebs, Andrea Costantini, and Jürgen Junk. Additionally, to the friends I had the pleasure of making in Luxembourg: Gabriela Junqueira, Carlos Wetzel, Demetrio Mora, and Boris König. I miss our laughter and our mix of Portuguese, English, Spanish, and German.

Ao Prof. Acácio T. Moreira de Melo e ao Eng. Florestal Ernesto Suzuki, bem como a Cooperativa Agrícola Mista de Tomé-Açu (CAMTA) pelo apoio e pela oportunidade única de usufruir do projeto “SAF Dendê” em nossa pesquisa. Estendo também meus agradecimentos aos professores Maurício R. Cherubin e Carlos E. P. Cerri pelo suporte através do RCGI e do CCARBON.

À Divisão de Atendimento à Comunidade (DVATCOM) da ESALQ/USP pela possibilidade de morar na Vila PG (residência estudantil) por mais de 1 ano. Isso foi crucial para minha permanência.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pela concessão da bolsa no país (Processos Nº 2022/10276-2) e no exterior (Processo Nº 2023/06313-2). À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001 pela bolsa de mestrado nos primeiros meses de curso.

Ao Programa de Pós-Graduação em Solos e Nutrição de Plantas (PPGSNP), em nome do Prof. Tiago Osório e da servidora Flávia Morales, e ao Departamento de Ciência do Solo, pela infraestrutura e recursos humanos necessários à realização do mestrado. Sou muito feliz por ter feito parte da ESALQ e da Universidade de São Paulo (USP), patrimônios genuínos da sociedade brasileira.

Aos demais membros da minha família por sempre estarem com os dedos cruzados por mim. Nós e o restante do povo pobre da periferia precisamos aprender a sonhar mais e assim transpor as determinações sociais que nos são impostas.

À cada trabalhador e trabalhadora que financiou a obtenção de mais esse grau acadêmico em uma universidade pública. Jamais esquecerei da dívida que tenho com vocês!

Finalmente, a todos, todas e todes que, direta e/ou indiretamente, contribuíram para o desenvolvimento desta pesquisa e que não tive condições de mencionar.

Muito obrigado / Thank you so much!

BIOGRAPHY

Alberto Vinicius Sousa Rocha é paraense, natural de Capitão Poço (estado do Pará, cerca de 216 Km da capital Belém) e está neste plano desde as ~05h:06min do dia 28 de dezembro de 1998. Aos 2 anos de idade, mudou-se para a cidade de Castanhal (estado do Pará, cerca de 76 Km da capital Belém) acompanhado de sua mãe, Alda Sousa, sua irmã, Jéssica Rocha, e pouco tempo depois, de sua avó, Maria de Nazaré. Na cidade de Castanhal, cumpriu a maior parte de seus graus escolares, incluindo o ensino primário (2012), o ensino médio (2016) e, finalmente, a graduação em Agronomia (2022). Em meados de 2019 desenvolveu uma paixão pela ciência, em particular pela ecologia do solo devido aos livros de Ana Maria Primavesi, e desde então investe suas energias a pesquisa científica baseada em sistemas. No ano de 2022 mudou-se para Piracicaba (estado de São Paulo, cerca de 156 Km da capital São Paulo e 2672 Km de Castanhal) para viver uma das suas maiores aventuras até aquele tempo: a pós-graduação em nível de mestrado. Com base em muito esforço (coletivo e pessoal) e nas oportunidades que lhe foram dadas, Vinicius Rocha foi o primeiro de sua família a alcançar e vivenciar certas coisas da vida; contudo, para ele, o mais importante não é ser o primeiro, mas garantir que não seja o último. Gosta de refletir sobre o mundo, a sociedade e as relações humanas. É um paraense apaixonado pela Amazônia e pela sua cultura. Quase foi cientista social e nas horas vagas assiste Friends e Brooklyn 99.

“Mistura de raça, dá loira, dá índia, morena
Meu povo vem ver as coisas do meu Pará
A minha cidade é linda é mais que um poema
Me orgulho em dizer que isso é Belém
É Belém do Pará, Carimbó, Síria, Tucupi, Tacacá, Açaí na tigela
É Belém de "Fafá", Baía do Guajará, Ilha do Marajó, ai que coisa mais bela!
Eu vim de lá, eu vim de lá, eu vim de lá também
Eu vim de lá, eu vim de lá, do meu Pará-Belém”.

Calypso (Pará Belém)

“Eles querem que alguém
Que vem de onde nóis vem
Seja mais humilde, baixe a cabeça
Nunca revide, finge que esqueceu a coisa toda”.

Emicida (Mandume)

SUMMARY

RESUMO

Necromassa microbiana, carbono e agricultura: combinando microrganismos vivos e mortos para revelar a dinâmica do carbono sob agrofloresta na Amazônia

Os microrganismos do solo são reportados como mediadores e, mais recentemente, também contribuintes para a persistência do carbono orgânico do solo (COS) por meio da necromassa microbiana. No entanto, fatores como clima, uso da terra e *microbial traits* (atividade e diversidade) influenciam fortemente os ciclos de vida e morte microbiana e, portanto, o acúmulo de C derivado de microrganismos, principalmente em regiões tropicais. Desse modo, usamos parcelas replicadas de dendê (*Elaeis guineensis*) em um sistema agroflorestal a base de dendê (SAF) e em monocultura de dendê (doravante "agricultura convencional", AC) na Amazônia brasileira para investigar os impactos do uso da terra (manejo agrícola), profundidade do solo e sazonalidade na atividade enzimática extracelular e no pool de C da necromassa microbiana. Além disso, também avaliamos os conteúdos de C nas frações físicas da matéria orgânica do solo (MOS, C-MOP e C-MOAM) e na montagem de comunidades bacterianas e fúngicas do solo associadas a cada uso da terra. Coletivamente, nossos resultados demonstraram que a adoção de SAF, baseado na diversificação da cobertura do solo, aumenta significativamente o COS, C-MOP, C-MOAM e a atividade de enzimas relacionadas aos ciclos C (β-glicosidase, em geral), S (arilsulfatase) e parcialmente P (fosfatase ácida) em comparação com AC ao longo do perfil vertical do solo. Também promoveu o conteúdo de necromassa fúngica e total, mas não necessariamente necromassa bacteriana, com o volume de necromassa sendo dominado pela necromassa fúngica. Curiosamente, a razão necromassa microbiana:SOC foi menor em AFS e/ou igual a CA e às vezes aumentou com a profundidade do solo (o máximo contabilizado foi ~28%). Além disso, a composição, riqueza e diversidade de comunidades microbianas (bactérias e fungos) foram montadas de acordo com o uso do solo e promoveram efeitos importantes no funcionamento do solo. Por fim, C-POM, C-MAOM e a atividade da β-glicosidase e arilsulfatase, bem como os índices de diversidade fúngica, Ca^{2+} trocável e conteúdo de argila foram os atributos mais importantes para predizer a necromassa. Nossas descobertas mostraram um papel crítico da cobertura da terra na dinâmica do C do solo e destacam o efeito real da agrofloresta na promoção do C total e de formas funcionalmente distintas de C na matéria orgânica, bem como *traits* microbianas específicas. Além disso, fornecem os primeiros insights sobre os caminhos do acúmulo de C de necromassa em sistemas agrícolas na Amazônia brasileira.

Palavras-chave: Sistemas agroflorestais, cultivo de dendê, carbono orgânico do solo, necromassa microbiana, comunidades microbianas do solo

ABSTRACT

Microbial necromass, carbon and agriculture: combining living and dead microbes to reveal carbon dynamics under agroforestry in the Amazon

Soil microbes are reported to mediate and, more recently, also contribute to soil organic carbon (SOC) persistence through microbial necromass. However, factors such as climate, land use, and microbial attributes (activity and diversity) strongly influence microbial life and death cycles and therefore the accumulation of microbial-derived C, mainly in tropical regions. Thereby, we used replicated oil palm (*Elaeis guineensis*) plots in an oil palm-cropland agroforestry system (AFS) and in oil palm monoculture (hereafter “conventional agriculture”, CA) in the Brazilian Amazon to investigate the impacts of land use (agricultural management), soil depth, and seasonality on extracellular enzymatic activity and the microbial necromass C pool. In addition, we also assessed C contents in the physical fractions of soil organic matter (SOM) (C-POM and C-MAOM) and on the assembly of soil bacterial and fungal communities associated with each land use. Collectively, our results demonstrated that the adoption of AFS, based on land cover diversification, significantly increases SOC, C-POM, C-MAOM and the activity of enzymes related to the C (β-glucosidase, in general), S (arylsulfatase) and partially P (acid phosphatase) cycles compared to CA along the vertical soil profile. It also promotes the content of fungal and total necromass, but not necessarily bacterial necromass, with the volume of necromass being dominated by fungal necromass. Interestingly, the microbial necromass:SOC ratio was lower in AFS and/or equal to CA and sometimes increased with soil depth (maximum accounted was ~28%). Moreover, the composition, richness and diversity of microbial communities (bacteria and fungi) were assembled according to land use and promoted important effects on soil functioning, including C fluxes. Lastly, C-POM, C-MAOM and the activity of β-glucosidase and arylsulfatase, as well as fungal diversity indices, exchangeable Ca^{2+} and clay content were the most important attributes to predict necromass. Our findings showed a critical role of land cover in soil C dynamics and underline the real effect of agroforestry on promoting total C and functionally distinct forms of C in organic matter, as well as specific microbial traits. In addition, they provide the first insights into the pathways of necromass C accumulation in agricultural systems in the Brazilian Amazon.

Keywords: Agroforestry systems, oil palm plantation, soil organic carbon, microbial necromass, soil microbial communities

1. INTRODUCTION

Soil organic carbon (SOC) management is crucial to land-based efforts to mitigate carbon emissions, sequester atmospheric carbon dioxide and deliver ecosystem services (Bossio et al., 2020). In the framework of nature-based strategies to preserve and enhance soil carbon for sustaining food production, agroforestry systems (AFS) emerge as one of the most promising opportunities (Beillouin et al., 2023). AFS are land use and management systems characterized by intensive, diverse practices and high ecological interaction between agricultural and forest crops (Nair, 2017; Visscher et al., 2024). According to global estimates, multi-strata AFS (systems with structural, spatial and vegetation layer diversity, as well as perennialism and niche complementarity) (Nair, 2017) can increase SOC by +30% compared to conventional agricultural practices and provide substantial carbon sequestration up to 0.31 Pg C yr^{-1} (Beillouin et al., 2023; Terasaki Hart et al., 2023).

In Brazilian Amazon, AFS have been recommended as productive, resilient, and sustainable strategies for oil palm (*Elaeis guineensis*) plantation as compared to traditional monocultures (hereafter “conventional agriculture”, CA) (Gomes et al., 2021), which historically developed from the deforestation of primary or secondary rainforests (Benami et al., 2018). The advantages of land use changing from CA to oil palm AFS include improvement in soil physical (aggregation and soil moisture content) (Oliveira et al., 2022a) and chemical properties (contents of Mg, K and P) (Costa et al., 2023), as well as biological attributes (microbial traits), including increased microbial biomass, activity and diversity (Visscher et al., 2024). Thus, supporting unique microbiomes and potentially determining ecological strategies based on distinct key traits for soil microbes (Leite et al., 2023; Malik et al., 2020).

However, although evidence shows that increasing plant species diversity can benefit SOC (Chen et al., 2020; Lange et al., 2015; Qian et al., 2023), it is still unclear how the diversification of plant biomass (below and aboveground) and therefore root exudation (quantitative and qualitative), can shape the dynamics of C stabilization in the soil under CA and AFS, especially in the more persistent pools. These pools may be dominated by microbial necromass (products and residues of biomass, such as dead cells, cell parts, cellular debris, and extracellular polymeric substances), which progressively accumulates in the soil after microbial life and death cycles (Buckeridge et al., 2022). The conceptual framework for this process is termed the "microbial

carbon pump" (MCP) (Liang et al., 2017) and supports global microbial contributions of between 15 and 80% to SOC, depending on the type of environment and land cover (forest, grassland, or cropland) (Bai & Cotrufo, 2022; J. Hu et al., 2023; Zhou et al., 2023).

Nevertheless, some geographic regions and biomes of the world remain unrepresented (or underrepresented) and microbial-derived C content is still poorly understood, especially in tropical regions (Beidler et al., 2020; J. Hu et al., 2023), including the Amazon rainforest. The global lack of knowledge exists because the accumulation of microbial necromass (bacterial and fungal) is influenced by many factors, such as microbial activity and diversity, establishment time and type of agricultural practice, as well as available C chemistry and environmental conditions (Creamer et al., 2019; J. Hu et al., 2023; Kallenbach et al., 2016). These factors may also influence the contents of C present in the physical fractions of soil organic matter (SOM), which can be subdivided, according to its functionality and persistence, into the relatively labile particulate organic matter (POM) and more persistent mineral-associated organic matter (MAOM) (Lavallee et al., 2020).

The contents present in those physical SOM pools can be used as reliable indicators to inform soil carbon sequestration under different land management regimes, as they generate insights into the quality of SOM (origin, lability, and persistence) rather than only the quantity present in the soil (Cotrufo et al., 2019). Thereby, research approaches that integrate aboveground diversity with belowground microbial traits and their interaction with environmental conditions in long-term systems are useful to provide a more complete overview of ecosystem-level carbon fluxes in soils under AFS and CA. This approach is particularly relevant for the Amazon, a hotspot for agroforestry in Brazil that hosts about 90% of the national oil palm production and faces constant agricultural and environmental pressure (Artaxo, 2023; Benami et al., 2018; Gomes et al., 2024). Thus, to address these knowledge gaps, we conducted a comprehensive field study to investigate the impacts of long-term adoption of multi-stratum AFS (mix between oil palm, regional Amazonian agricultural crops and trees) in comparison to CA and the extent of these changes in attributes core to C storage and quality.

Our main objectives were: (i) to reveal how above-ground plant biomass diversification can shape the microbial activity and diversity of fungi and bacteria (traits), and C microbial necromass (bacterial and fungal) as a proportion of SOC, (ii)

to evaluate whether the C contents presented in the SOM pools (POM and MAOM) in the different agricultural regimes can be used as an indicator of C storage in each land use and, finally, (iii) to explore the influence of environmental factors (e.g., soil depth, physical-chemical attributes and seasonality) on the dynamics of assessed C pools (microbial, total and in physical) in tropical Amazonian oil palm soils.

2. MATERIALS AND METHODS

2.1. General characteristics of the study sites

The study was conducted with soils from the municipality of Tomé-Açu (PA), located in the Amazon biome and the Northern region of Brazil (Lat.: 02° 40' 54" S; Long.: 48° 16' 11" W, 55 m.a.s.l.) (Figure 1a). The predominant soil in this region is classified as Yellow Ferralsols (FAO taxonomy, equivalent to Yellow Oxisols in U.S.A. Soil Taxonomy), and the climate is classified as Ami (Köppen), hot and humid. The average annual temperature is 26 °C and the relative humidity is 85%, with an average annual precipitation of 2,300 mm, occurring mainly from December to May. In general, soils in this region are considered to have high acidity and low natural fertility.

2.2. Soil management systems

Soil samples were collected in September 2022 and January 2023 (dry and rainy seasons, respectively) from two land use systems. The management systems in the first site consisted of an oil palm (*Elaeis guineensis*) plantation established in 2003 (Lat.: 2° 22' 11.54" S; Long.: 48° 15' 24.60" W) and managed under conventional agriculture (CA) practices, characterized by monoculture and regular applications of synthetic fertilizers and pesticides. In contrast, the second site featured an agroforestry system (AFS) based on oil palm established in 2008 (Lat.: 2° 20' 56.84" S; Long.: 48° 15' 40.90" W) and managed using more sustainable practices, organic fertilization, maintenance of soil cover and no-tillage. Besides oil palm, the AFS also included Amazonian agricultural crops, such as cocoa (*Theobroma cacao*), açaí (*Euterpe oleracea* Mart.) and tree species planted in strips (hereafter “strip under diversification and integration”, SID) interspersed between the oil palm rows, belonging to the experimental area "Oil palm-based agroforestry in family agriculture” (Carvalho et al., 2014). For details on crop management and employment of agricultural inputs, refer to historical series available in Table S1.

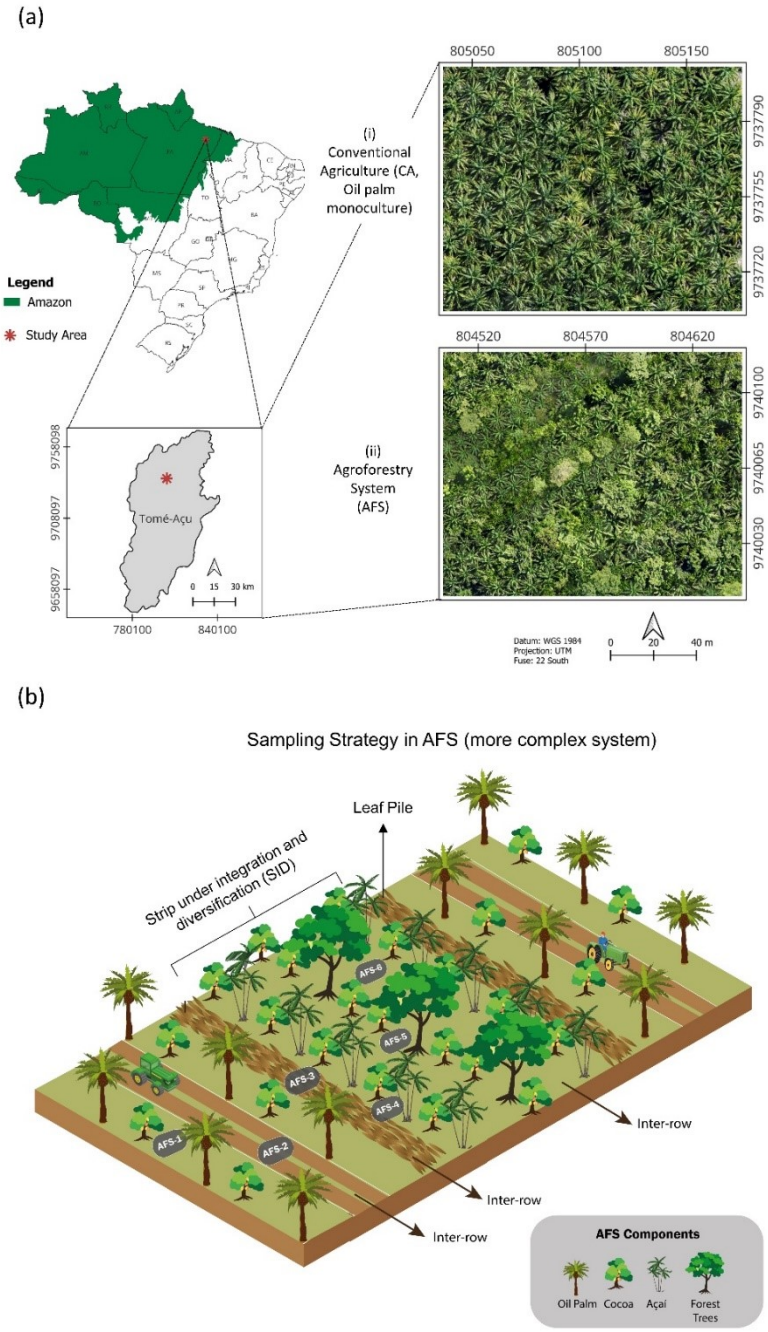

Figure 1. Location map of the study sites at (a) macro and micro scales, containing (i) monoculture oil palm plantation (CA) and (ii) agroforestry system (AFS) with oil palm as the main crop; and (b) structural organization of the AFS and the adopted sampling strategy (shaded in gray and ranging from AFS-1 to AFS-6) to incorporate plant interactions in the assessments conducted in this study in the municipality of Tomé-Açu, state of Pará, Eastern Amazon, Brazil.

2.3. Experimental design and sampling strategy carried out

From each land use (CA and AFS), the litter layer was removed and then composite samples (formed by 3 simple samples) were collected in each of 5 spatially

replicated plots in rows and inter-rows of each agricultural and/or forest crop, at 3 different depths (0-10, 10-20, and 20-30 cm) and during 2 seasons. This resulted in 8 sampling points (2 in CA and 6 in AFS, Table 1) and a total of 240 samples (8 sampling points × 2 seasons × 3 depths × 5 sampling replicates). Despite its complexity, this sampling strategy was adopted with the aim of establishing a similar procedure for the assessed systems and understanding how management zones (within-row and between-row in plantations), plant type (agricultural (e.g., local or exotic) or tree), and the associated vegetative stratum could impact the evaluated parameters (Table 1).

The vegetative stratum becomes relevant for the reason that each plant present in the crops (agricultural or tree) possesses a specific genotype, with variations in above and below-ground carbon input (e.g., rhizodeposits, dissolved organic carbon from dead roots and litter leachate, and litter deposition on the surface) and the capacity to form unique ecological relationships with the associated microbial community (Carvalho et al., 2023; Mendes et al., 2013; Sokol et al., 2019).

Table 1. Description of the 8 sampling points and their respective interactions within the 2 assessed land uses (CA and AFS), along with the assigned acronyms employed across this study.

Land use	Acronym	Sampling Point
CA	CA-1	Plantation row
	CA-2	Plantation inter-row
AFS	AFS-1	Oil palm + cocoa plantation row
	AFS-2	Oil palm + cocoa plantation inter-row
	AFS-3	Inter-row space between the oil palm + cocoa plantation row (AFS-2) and the açaí + cocoa plantation row
	AFS-4	Açaí + cocoa plantation row
	AFS-5	Tree + cocoa plantation row
	AFS-6	Inter-row space between the tree + cocoa plantation row (AFS-5) and the açaí + cocoa plantation row (AFS-4)

*CA: conventional agriculture; and AFS: agroforestry system.

2.4. Analyses of the physicochemical attributes

The following chemical attributes were determined: pH in a 1:2.5 soil/water suspension, and exchangeable Ca^{2+} and Mg^{2+} were extracted with 1M KCl. Ca^{2+} and Mg^{2+} were determined by atomic absorption spectrometry. K^{+} was extracted by ion-exchange resin and potential acidity (H + Al) was estimated by an equation based on the pH determined in SMP buffer solution (pH SMP). Ca^{2+}, Mg^{2+}, K^{+}, and H+Al are reported as mmolc/dm³. Regarding physical parameters, the following were evaluated:

moisture (oven-drying method) and soil texture (Bouyoucos hydrometer method): sand (sieve analysis), clay (sedimentation), and silt (1000 – (clay + sand)). We emphasize, however, that soil texture assessment (reported as %) was conducted only for samples collected during the rainy sampling season. We made this decision under the premise that texture is relatively stable and exhibits little to no short- and medium-term temporal variability. Therefore, we chose to extrapolate the data obtained during the rainy campaign, during which we had a larger volume of soil available, also for the dry season (soil properties are provide in Table S2).

2.5. Soil microbial activity

The potential activity of the extracellular enzyme β-glucosidase, arylsulfatase, and acid phosphatase were assayed and used as indicators of soil microbial activity. To do this, the method described by Tabatabai (1994) was adopted with some modifications. Briefly, ~1.0 g of fresh soil was placed into 10 mL test tubes, followed by the addition of 4 mL of MUB buffer solution (pH 6.0 for β-glucosidase and pH 6.5 for acid phosphatase) or acetate buffer (pH 5.8 for arylsulfatase). Afterwards, the solutions containing soil were shaken and incubated at 37 °C for 1 h with 50 mM p-nitrophenyl buffer solution (p-nitrophenyl-β-d-glucopyranoside for β-glucosidase, p-nitrophenyl potassium sulphate for arylsulfatase, and p-nitrophenyl phosphate for acid phosphatase) (Sigma Aldrich, St. Louis, MO, U.S.A). The quantification of soil enzymatic activity was based on the colorimetric determination of p-nitrophenol released (420 nm for β-glucosidase and 410 nm for arylsulfatase and acid phosphatase).

2.6. Soil organic matter (SOM) physical fractionation

The samples were physically fractionated into mineral-associated organic matter (MAOM) and particulate organic matter (POM) using the particle size method proposed by Cambardella & Elliott (1992). Specifically, ~5 g of sieved soil (< 2 mm) was weighed into a 50 mL tube with 1 glass marble and 15 mL of sodium hexametaphosphate solution (5 g L^{-1}) and dispersed on a horizontal shaker for 16 hours at 140 rpm. The dispersed solution was then passed through a 53 μm sieve

while gently adding a stream of deionized water. The coarse fraction (> 53 µm, POM) retained on the sieve and the washed material (< 53 µm, MAOM) were stored, dried in an oven (50 °C), finely ground (<100 µm), and had their C content determined using on a LECO CN-2000. Based on the results, it was also possible to calculate the total C pool present in the SOM in the bulk soil.

2.7. Extraction and determination of amino sugars in soil samples

The extraction of the amino sugars Glucosamine (GluN) and Muramic Acid (MurA), considered as biomarkers for microbial necromass, was based on the method developed by Appuhn et al. (2004) - with small modifications. To achieve this, 500 mg of sieved (<2 mm) and air-dried soil (bulk soil) was immersed in 10 mL of HCl (6 M) and hydrolyzed for 6 hours at 105°C. After reaching room temperature, the samples were shaken, and a 1 mL aliquot was extracted and filtered through a PTFE membrane (0.22 µm). Subsequently, to remove the HCl, a 500 µL aliquot was evaporated to dryness under a nitrogen atmosphere (40-45°C), reconstituted in 1 mL of ultrapure water, evaporated a second time under similar conditions, and reconstituted in 500 µL of ultrapure water. Then, the samples were subjected to an ultrasonic bath for 5 seconds, with the aim of homogenizing the samples, and stored at 4°C until analysis (≤ 1 day).

For the determination of the target compounds, High-Performance Liquid Chromatography (HPLC) was employed following Indorf et al. (2011). Briefly, analyses were conducted on an Agilent 1200 HPLC system fitted with a Phenomenex (Aschaffenburg, Germany) Hyperclone C-18 (ODS) column (125 mm length × 4 mm diameter, 5 µm particle size, 12 nm pore size), and a fluorescence detector (445 nm emission and 330 nm excitation). In the pre-derivatization process, 500 µL of ortho-phthaldialdehyde (OPA) and 300 µL of the obtained sample were combined in 2 mL vials and injected into the HPLC system after approximately 120 seconds of reaction time. Analytical standards (Sigma Aldrich, St. Louis, MO, U.S.A.) used included D-(+)-glucosamine hydrochloride and muramic acid. For additional information regarding the run time, flow rate, system temperature, and mobile phase characteristics (among others), refer to Indorf et al. (2011).

2.8. Estimation of microbial C necromass and contribution to SOC

The C of microbial necromass was estimated following the stoichiometric bases delivered by Joergensen (2018) and Liang et al. (2019), which take into account the contents of GluN and MurA. Hence, fungal necromass (FN), bacterial necromass (BN), and total microbial necromass C (TMN) were calculated as follows:

i. Content of fungal necromass (FN) =

$$\left(\frac{GluN\ (mg.g^{-1})}{179.17} - 2 * \frac{MurA\ (mg.g^{-1})}{251.23}\right) * 179.17 * 9$$

ii. Content of bacterial necromass (BN) = $MurA * 45$

iii. Content of total microbial necromass (TMN) = $NF + NB$

Where 179.17 and 251.23 are the molecular weights of GluN and MurA, respectively; 9 is the conversion factor from GluN to fungal necromass; and 45 is the conversion factor from MurA to bacterial necromass. Then, based on the obtained C contents, the percentage contribution of microbial residues to SOC was calculated.

2.9. DNA extraction, sequencing, and bioinformatics data processing

DNA extraction was carried out from 0.25 g of frozen soil (-80 ºC) using the DNeasy PowerSoil Pro Kit (Qiagen, Germany), according the manufacturer's protocol. The quality indicators of the DNA were assessed on a NanoDrop 2000c (Thermo Fisher Scientific, Inc.) and the samples stored at -80°C until further analyses. Amplicon sequencing, in turn, was performed on a MiSeq (Illumina Inc.) through amplification of the V4 region of the 16S rRNA gene for bacteria with the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'); and the ITS region for fungi with the primers ITS1-1F-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS1-1F-R (5'-GCTGCGTTCTTCATCGATGC-3'). This process was carried out only for the samples from the 0-10 cm depth and in four replicates, as the assessment of the topsoil layer was ecologically more relevant in this study and also as a strategy to reduce the large number of samples to be analysed.

The sequences obtained from 16S rRNA and ITS were processed using R (version 4.2.3) (R Core Team, 2023) via the R Studio interface (version 2023.12.0.369)

(RStudio Team, 2023) according to DADA2 pipeline (Callahan et al, 2016). To summarize, the demultiplexed 16S rRNA and ITS sequences had their barcodes and primers removed. Primers were removed using the *trimLeft()* function for bacteria and *Cutadapt* for fungi (Martin, 2011). Quality control was then applied using the consensus method to remove any remaining chimeric and low-quality sequences (only samples with QScore > 25 were kept for bacteria, and the average sequence length was maintained around 170 nts for fungi). Afterwards, the samples were paired, rarefied to 31,839 and 53,300 sequences (following the number of the lowest sample) for bacteria and fungi (respectively), and designated as Amplicon Sequence Variants (ASVs) at 99% similarity. The taxonomic classification for bacteria was done through the SILVA database (version 138.1) (Quast et al., 2012) and for fungi using the UNITE database (10.0 version) (Nilsson et al., 2019).

2.10. Data analysis

Before interrogating the data, measured variables were assessed for normality using the Shapiro-Wilk test and, where necessary, outliers were removed, and data were natural log transformed + 1 to approximate assumptions of normality (1 was a constant to avoid negative values). Next, to appropriately represent the effects of 8 sample points collected within the 2 land uses (2 in CA and 6 in AFS) and seasonal variation (dry and rainy), a linear mixed effects model (LMM) was employed to soil enzymatic activity (β-glucosidase, arylsulfatase, and acid phosphatase), necromass contents (fungal and bacterial), and SOM carbon pools (C-POM, C-MAOM, and total SOC), once that the experimental design was not completely independent/random (Slaets et al., 2021). In the model, the fixed effects included sampling points and season, while the random effect was the plot, aiming to account for data variation related to specific differences within each plot.

The analysis was carried out separately for each soil depth (0-10, 10-20, and 20-30 cm) and the effect of the season within and between sample points was assessed by multiple comparisons using the Tukey post-hoc test ($p \leq 0.05$) (Hothorn et al., 2008). The decision to run the model for each soil layer was based (a) on the complexity and potential loss of statistical power that the inclusion of a third factor of variation (beyond the sampling point and seasonality of precipitation) would introduce into the model; and (b) on the natural environmental differences existing along the

vertical soil profile (e.g., greater influence of plant root systems, diversity of C sources, and higher microbial activity on the upper layers), which are generally decoupled from agricultural management. Thereby, drawing comparisons between soil depths was not ecologically relevant for us and we therefore chose to adopt a stratified approach focusing on the environmental conditions inherent in the soil system.

Regarding DNA sequencing data, alpha diversity (Shannon and Simpson indices) was calculated through *phyloseq* package and was also analyzed using an LMM followed by a Tukey post-hoc test ($p \leq 0.05$) as the same conditions described above. Soil beta diversity was assessed by Principal Coordinate Analysis (PCoA) using Bray-Curtis distance matrices (9999 permutations) and a permutation multivariate analysis of variance (PERMANOVA) was employed to verify the effect of sampling points and seasonality on microbial groups within CA and AFS. Distance-based redundancy analysis (dbRDA) of Bray-Curtis dissimilarity matrices was used to identify the determinants factors in the structuring of both bacterial and fungal communities (only highly significant variables ($p \leq 0.05$) were included in the model). Complementarily, Mantel tests were conducted to test relationships between the variation of community composition (Bray-Curtis distance) and the variation in measured variables (Euclidean distance) to reveal the driving forces shaping the microbial community associated with each land use.

In addition, boosted regression tree analysis (learning rate = 0.001) (Elith et al., 2008) was performed to investigate the relative importance of the microbial traits (soil enzymatic activity and fungal and bacterial diversity indices) and the soils physical and chemical attributes in determining the variation of BN and FN in the upmost soil layer (0-10 cm) via *dismo* (Hijmans & Phillips, 2023) package in R. At last, Spearman's correlation was employed to explore the relationships among biological, physical, and chemical attributes on our study (also at 0-10 cm depth). All models and analyses were fitted in R programming (version 4.2.3) through the R Studio interface (version 2023.12.0.369) using, in addition to the packages mentioned above, the following libraries: *Biostrings* (Pagès et al., 2022), *car* (Fox & Weisberg, 2019), *emmeans* (Lenth, 2024), *factoextra* (Kassambara & Mundt, 2020), *FactoMineR* (Lê et al., 2008), *file2meco* (Liu et al., 2022), *metan* (Olivoto & Lúcio, 2020), *multcomp* (Hothorn et al., 2008), *nlme* (Pinheiro & Bates, 2023), *openxlsx* (Schauberger & Walker, 2023), *patchwork* (Pedersen, 2024), *readxl* (Wickham & Bryan, 2023), *rstatix* (Kassambara, 2023), *tidyverse* (Wickham et al., 2019), and *vegan* (Dixon, 2003).

3. RESULTS

3.1. Influence of land use on soil microbial activity

The potential activity of β-glucosidase, arylsulfatase, and acid phosphatase partially differed between land uses (as revealed by the distributed sampling points within the CA and AFS) and the above-ground biomass played a key role on soil microbial activity (between and within land use) at each soil depth. For the 0-10 cm layer, in general, β-glucosidase was higher (over 300%, ranging from 11.2 6 ±1.48 to 33.09 ±1.21 mg PNF.g $^{-1}$ soil hour $^{-1}$) at sampling points across AFS compared to points within CA ($p \leq 0.05$), mainly in the strip under integration and diversification (SID) (AFS-3 to AFS-6, Table 2). This pattern was also true for arylsulfatase, which expressed greater activity in AFS than CA (up to 8 times more, ranging from 78.12 ± 17.91 to 643.25 ± 18.48 mg PNF.g $^{-1}$ soil hour $^{-1}$) in most comparisons among the 8 sampling points and had it is performance increased in the rainy season. Acid phosphatase at 0-10 cm depth, in turn, had its activity strongly shifted by the season and did not appear to be a sensitive indicator to differentiate managements treatments under the experimental conditions investigated.

At other soil layers (10-20 and 20-30 cm), AFS and CA showed few differences between them for the activity of β-glucosidase, with greater enzymatic potential in the dry season, in which CA-1 presented the highest values at 20-30 cm layer (ranging from 25.99 ± 1.96 to 8.65 ± 1.83 mg PNF.g $^{-1}$ soil hour $^{-1}$) in compare to the sampling point with the lowest activity in AFS. On the other hand, arylsulfatase and acid phosphatase had their measurements increased in most sampling points located in AFS than in the points within CA (with a promoting effect during the rainy season), indicating the potential of agroforests to act as mitigating the effects of agricultural practices also in deeper layers of the soil.

Table 2. Potential activity of extracellular enzymes β-glucosidase, arylsulfatase, and acid phosphatase according to the 8 sampling points (2 in CA and 6 in AFS) and seasonality. Upper letters within each plot indicate a significant difference between the dry and rainy seasons, and lower-case letters indicate a significant difference between sampling points within each season according to Tukey's test ($p \leq 0.05$). Values are reported as mean ± standard error (SE) (n = 5).

Land Use	Sampling Point	β-glucosidase		Arylsulfatase		Acid Phosphatase	
		Dry	Rainy	Dry	Rainy	Dry	Rainy
		mg PNF.g $^{-1}$ soil hour $^{-1}$					
				Depth: 0-10 cm			
CA	CA-1	19.43 ± 2.74 Aabcd	11.26 ± 1.48 Bc	68.45 ± 20.93 Ac	78.12 ± 17.91 Ac	304.42 ± 34.38 Aa	339.15 ± 21.08 Ac
	CA-2	12.44 ± 0.88 Acd	14.83 ± 1.83 Abc	177.20 ± 28.03 Aab	216.50 ± 21.41 Ab	342.24 ± 83.84 Aa	406.41 ± 37.05 Abc
AFS	AFS-1	11.16 ± 1.21 Ad	18.44 ± 3.94 Abc	200.62 ± 35.40 Bab	332.81 ± 7.14 Aab	317.48 ± 24.80 Aa	388.85 ± 25.30 Abc
	AFS-2	12.91 ± 1.09 Acd	12.51 ± 2.54 Ac	111 ± 12.61 Bbc	387.96 ± 76.54 Aab	288.43 ± 34.44 Ba	629.10 ± 109.81 Aa
	AFS-3	17.02 ± 1.81 Abcd	20.50 ± 0.82 Aab	185.53 ± 32.34 Bab	418.60 ± 8.90 Aab	419.25 ± 18.13 Aa	462.93 ± 68.14 Aabc
	AFS-4	26.86 ± 1.20 Aab	25.47 ± 3.46 Aab	254.43 ± 32.92 Aa	392.26 ± 12.79 Aab	336.84 ± 20.17 Ba	564.25 ± 72.45 Aab
	AFS-5	30.84 ± 4.16 Aa	33.09 ± 1.21 Aa	305.54 ± 33.26 Ba	643.25 ± 18.48 Aa	344.27 ± 23.57 Ba	669.18 ± 39.05 Aa
	AFS-6	20.92 ± 2.68 Babc	35.49 ± 2.77 Aa	186.04 ± 19.41 Bab	534.05 ± 43.30 Aa	415.65 ± 26.70 Aa	462.70 ± 14.56 Aabc
				Depth: 10-20 cm			
CA	CA-1	17.83 ± 2.55 Aab	6.36 ± 0.99 Bbc	30.68 ± 7.04 Ab	12.94 ± 4.64 Ad	211.88 ± 6.01 Ab	259.78 ± 14.22 Ab
	CA-2	12.04 ± 0.89 Abc	8.84 ± 1.65 Abc	52.91 ± 7.36 Aab	92.22 ± 3.28 Abc	300.81 ± 30.47 Aab	292.93 ± 33.12 Ab
AFS	AFS-1	8.83 ± 0.97 Ac	6.29 ± 1.55 Abc	54.88 ± 2.90 Bab	178.39 ± 30.47 Aa	314.10 ± 8.18 Bab	532.78 ± 9.01 Aa
	AFS-2	10.57 ± 1.85 Ac	3.13 ± 1.27 Bc	66.65 ± 17.29 Aab	62.38 ± 24.77 Acd	411.37 ± 55.36 Ba	540.38 ± 39.04 Aa
	AFS-3	13.56 ± 2.61 Abc	6.69 ± 0.34 Bbc	73.40 ± 20.45 Bab	146.14 ± 10.55 Aab	448.29 ± 21.45 Aa	461.58 ± 38.66 Aa
	AFS-4	17.91 ± 1.44 Aab	12.79 ± 2.74 Aab	119.79 ± 11.68 Aa	146.70 ± 8.39 Aab	444.69 ± 41.50 Aa	501.83 ± 43.77 Aa
	AFS-5	21.82 ± 1.52 Aa	18.65 ± 1.66 Aa	104.98 ± 5.13 Bab	206.26 ± 19.86 Aa	386.82 ± 38.15 Ba	524.62 ± 44.04 Aa
	AFS-6	12.04 ± 0.77 Abc	3.10 ± 0.60 Bc	73.18 ± 22.93 Bab	135.97 ± 35.55 Aabc	420.60 ± 16.82 Aa	508.64 ± 57.18 Aa
				Depth: 20-30 cm			
CA	CA-1	25.99 ± 1.96 Aa	3.04 ± 0.89 Bb	31.62 ± 2.95 Ac	9.13 ± 0.54 Ab	215.59 ± 6.09 Ab	172.70 ± 47.64 Ac
	CA-2	9.90 ± 1.91 Abcd	6.47 ± 1.15 Aab	28.71 ± 6.41 Ac	42.30 ± 8.10 Aab	236.87 ± 34.68 Ab	196.11 ± 27.39 Acd
AFS	AFS-1	10.35 ± 1.39 Abcd	3.08 ± 1.17 Bb	25.54 ± 7.15 Bc	57.79 ± 4.53 Aa	365.21 ± 28.60 Bab	623.69 ± 71.04 Aa
	AFS-2	8.65 ± 1.83 Acd	2.82 ± 0.69 Ab	22.40 ± 7.94 Ac	44.11 ± 5.57 Aab	461.35 ± 21.00 Aa	365.04 ± 39.71 Abd
	AFS-3	14.99 ± 1.72 Ab	5.39 ± 1.00 Bab	46.72 ± 3.51 Aabc	36.30 ± 7.68 Aab	546.69 ± 14.37 Aa	495.80 ± 48.01 Aab
	AFS-4	13.70 ± 1.06 Abc	6.87 ± 0.90 Bab	87.25 ± 17.92 Aa	44 ± 9.38 Bab	403.49 ± 38.24 Bab	597.12 ± 62.39 Aa
	AFS-5	13.85 ± 0.63 Abc	9.51 ± 1.69 Aa	81.83 ± 14.52 Aab	71.92 ± 12.92 Aa	386.37 ± 38.09 Bab	537.91 ± 73.30 Aab
	AFS-6	7.38 ± 1.36 Ad	3.39 ± 1.27 Ab	41.84 ± 5.16 Abc	65.5 ± 13.70 Aa	534.76 ± 11.98 Aa	453.70 ± 40.70 Aab

*CA: conventional agriculture; and AFS: agroforestry system. The other abbreviations are explained in the “Methods section”, Table 1.

3.2. Microbial necromass C content and contribution to SOC

Small or no statistical differences were detected for bacterial content (BN) in our sites, indicating that the accumulation of bacterial residues in croplands is relatively stable across the sampling points distributed in CA and AFS at all soil depths ($p > 0.05$) (Figure 2a). On the other hand, fungal necromass (FN) tended to be more sensitive to land use conversion ($p \leq 0.05$) than BN and dominated the total microbial necromass (TMN) content (Figure 2b, c). Furthermore, the season proved to be an important driver in microbial residue concentration, as seen for FN and TMN, which showed greater differences between the groups measured in the dry period ($p \leq 0.05$). TMN levels, in turn, were generally higher in AFS than in CA (especially in SID, consisting of regional Amazonian species such as cocoa and açaí) at depths of 0-10 and 10-20 cm, ranging up to twice more between the highest and lowest sampling points in each land use. In opposition, this pattern was not true in the deeper layer (20-30 cm) and no major differences were expressed across the sampling points assessed (except to CA-1 and AFS-1). Finally, AFS-1 showed the lowest TMN values among all groups at both depths 10-20 and 20-30 cm during the dry season (1 ± 0.18 and 0.61 ± 0.05 $g.kg^{-1}$, respectively).

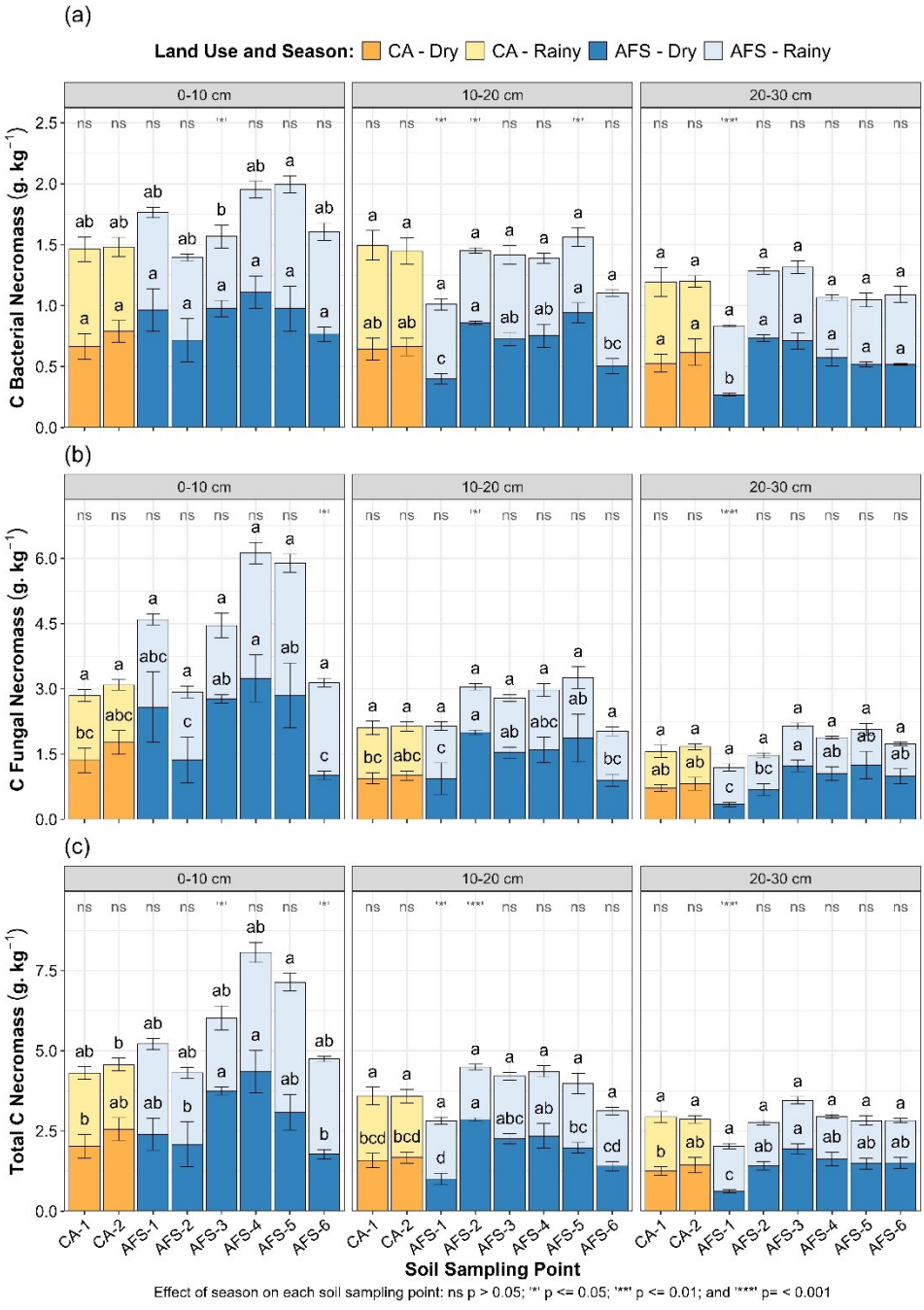

Figure 2. Contents of bacterial necromass (a), fungal necromass (b), and total necromass (c) according to the 8 sampling points (2 in CA and 6 in AFS), sampling season, and soil depth. Mean values followed by the same letter do not differ significantly among sampling points according to Tukey's test ($p \leq 0.05$). Significance codes for differences within sampling points due to sampling season: ns $p > 0.05$, '*' $p \leq 0.05$, '**' $p \leq 0.01$, and '***' $p \leq 0.001$. Values are reported as mean ± standard error (SE) (n = 5). Abbreviations are explained in the "Methods section", Table 1.

Notwithstanding the contents of BN and FN did not show significant differences between the studied sites, the contribution of each group to SOC varied from 2.7% to 11% for bacterial and from 4.5% to 19% for fungal necromass, reaching up to 27% of SOC for the total necromass along the soil depths (Figure 3). These variations were

dependent on the season and the type of land use assessed, according to the sampling points within the AFS and CA, but their interaction had minimal or no effects. We also realized that the proportion of microbial residues (BN, FN, and TMN) was statistically higher overall at the sampling points in CA compared to AFS, suggesting that soils under monoculture oil palm plantations have the capacity to accumulate proportionally more microbial-derived C than agroforestry system. Finally, although the comparison between soil depths was not the subject of our study, we observed a relative stability or even an increase in microbial contribution to SOC throughout the soil vertical profile (0-10, 10-20, and 20-30 cm).

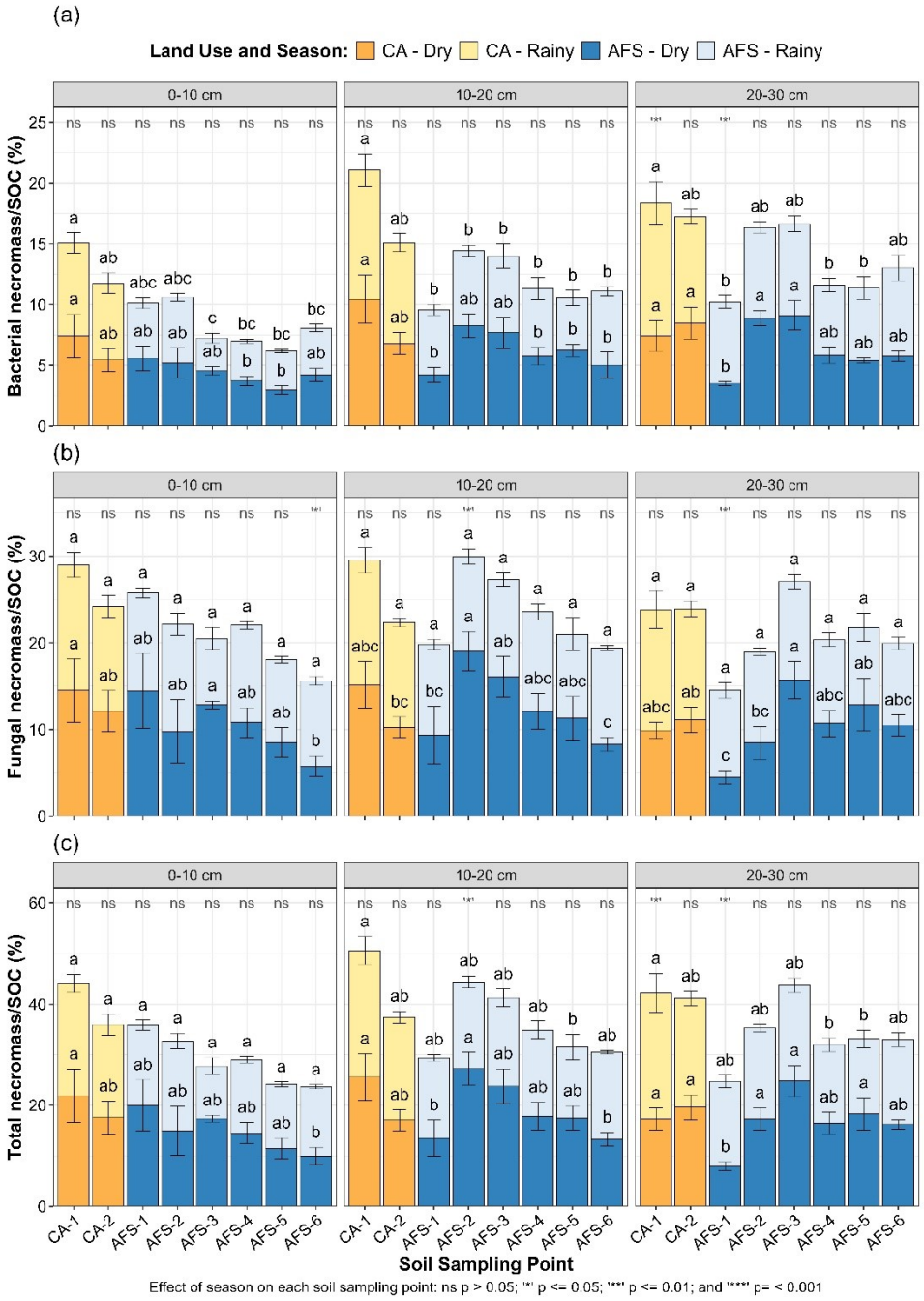

Figure 3. Proportion of bacterial necromass (a), fungal necromass (b), and total necromass (c) to SOC according to the 8 sampling points (2 in CA and 6 in AFS), sampling season, and soil depth. Mean values followed by the same letter do not differ significantly among sampling points according to Tukey's test ($p \leq 0.05$). Significance codes for differences within sampling points due to sampling season: ns $p > 0.05$, '*' $p \leq 0.05$, '**' $p \leq 0.01$, and '***' $p \leq 0.001$. Values are reported as mean ± standard error (n = 5). Abbreviations are explained in the "Methods section", Table 1.

3.3. Carbon pools in SOM physical fractions (POM and MAOM) as response to land use change

Changing land use from CA to AFS increased C contents in the particulate organic matter (POM), mineral-associated organic matter (MAOM), and SOC at most measured sampling points ($p \leq 0.05$). Overall, soil C levels was not shaped by variations in seasonality and decreased with soil depth (Figure 4). In the upmost layer (0-10 cm), soil carbon contents under AFS were up to 4 times higher in the POM (Figure 4a) and up to 200% more in the MAOM within the strip under integration and diversification (AFS-3 to AFS-6) compared to points located in CA (Figure 4b), with a similar pattern observed for SOC (Figure 4c). Conversely, minimal or no statistical differences were identified among the sampling points containing oil palm in both land uses (i.e., CA-1, CA-2, AFS-1, and AFS-2) to C-POM, C-MAOM and SOC, demonstrating a limitation in carbon accumulation under the cultivation of this plant.

In deeper soils (10-20 and 20-30 cm), land use changes also resulted in shift in soil C content, but to a lesser extent than those exhibited for 0-10 depth. To put it another way, there was relative stability in C levels in the measured forms (POM, MAOM and SOC) in each land use. Even so the sampling points distributed in AFS presented higher C level when compared to those located in CA ($p \leq 0.05$), including AFS-1 and AFS-2 (oil palm plantation). MAOM was the dominant pool of SOC compared to POM at our two sites and at three assessed depths. On average, the contribution of the pool associated with soil mineral surfaces was ~90% (ranging from ~79% to ~95%), while the more labile pool accounted for ~10% of SOC (ranging from ~5% to ~20%).

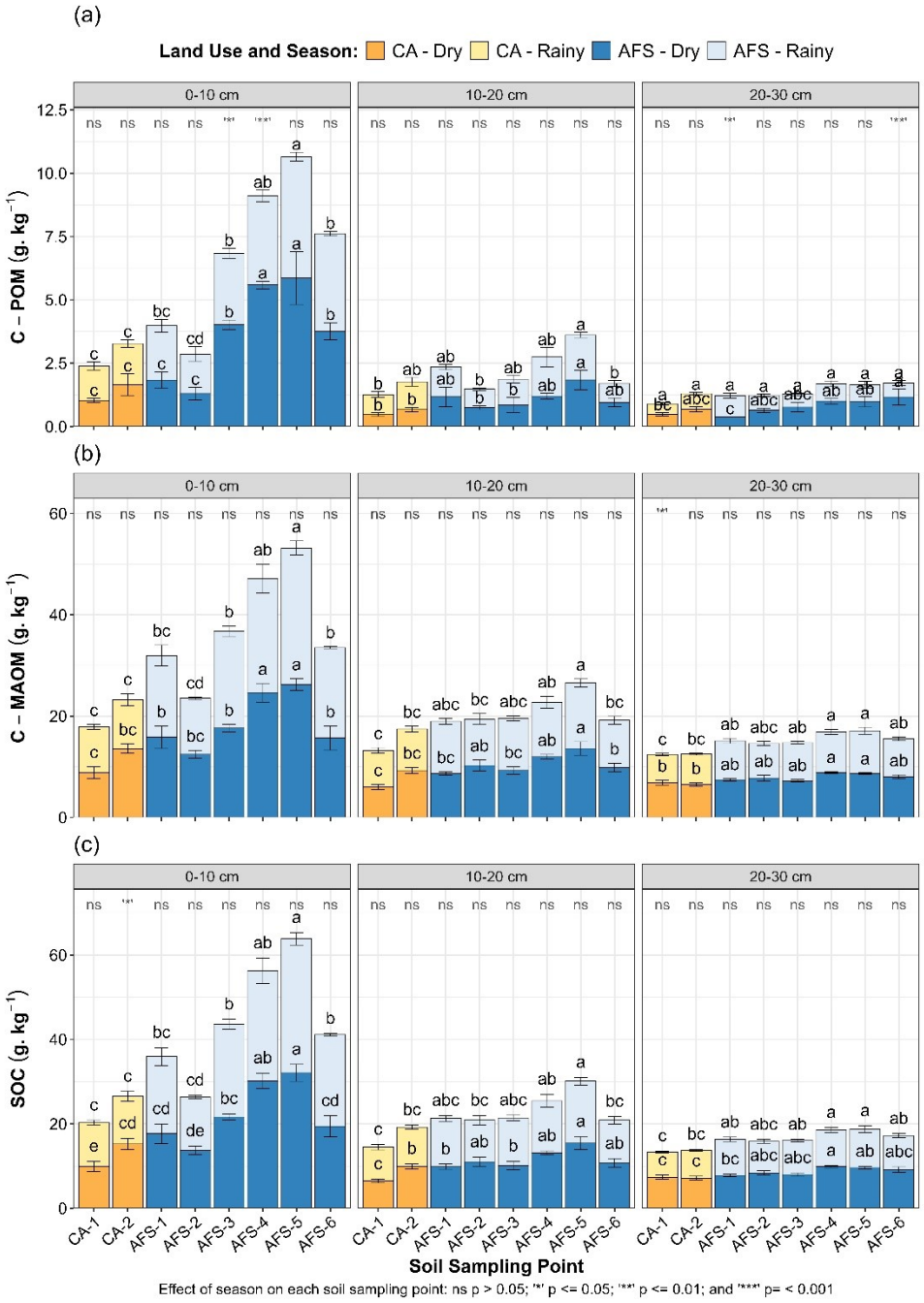

Figure 4. C contents presents in the particulate organic matter (POM) (a), mineral-associated organic matter (MAOM) (b), and total SOC according to the 8 sampling points (2 in CA and 6 in AFS), sampling season, and soil depth. Mean values followed by the same letter do not differ significantly among sampling points according to Tukey's test ($p \leq 0.05$). Significance codes for differences within sampling points due to sampling season: ns $p > 0.05$, '*' $p \leq 0.05$, '**' $p \leq 0.01$, and '***' $p \leq 0.001$. Values are reported as mean ± standard error (SE) (n = 5). Abbreviations are explained in the "Methods section", Table 1.

3.4. Effects of land use change on the assembly of bacterial and fungal communities

Based on a total of 4,534,200 high-quality reads (averaging 74,606 reads per sample), the taxonomic classification at the phylum level for bacteria showed the groups Proteobacteria (28%), Actinobacteriota (19.8%), and Acidobacteriota (12.9%), alongside Planctomycetota (11.5%) and Chloroflexi (6.77%) as the most abundant for both CA and AFS for the soil upmost layer (0-10 cm) (Figure S1a). For fungi, a total of 5,815,460 high-quality reads were analyzed (average of 92,876 reads per sample), revealing a community dominated by Ascomycota (53.7%), trailed by Basidiomycota (24.9%), Chytridiomycota (7.51%), Mortierellomycota (3.56%), and Rozellomycota (2.49%) (Figure S1b). Upon examining observed richness and alpha diversity measures, it was found that the number of bacterial ASVs was over 3 times higher than fungal ASVs, indicating a bacterial dominance in terms of richness (Figure 5a). Both bacterial and fungal richness were statistically higher at AFS sampling points compared to CA during the rainy season, except for fungi at AFS-1, AFS-2, and AFS-6. The Shannon and Simpson indices for bacteria and fungi did not exhibit significant differences between the two land uses investigated, not including CA-1 during the rainy campaign ($p \leq 0.05$) (Figure 5a).

The bacterial (Figure 5b) and fungal community (Figure 5c) structure across the sampling points differed significantly ($p \leq 0.05$), with an interaction between sampling point and season for bacteria. However, no interaction between sampling point and season was identified for fungal community. Despite the existing heterogeneity between and within the land uses investigated (as largely demonstrated in the previous sections), the PCoA and PERMANOVA analyzes clearly grouped the data set into two sample groups based in CA and AFS. However, it was still possible to observe for fungi a segregation across the oil palm sampling points (AFS-1 and AFS-2) in comparison to the others located in AFS (Figure S2b), which showed greater cohesion. Even so, differences in the structure of the bacterial and fungal communities were maintained in the oil palm plantation under both monoculture (CA-1 and CA-2) and agroforestry (AFS-1 and AFS-2) systems. This indicates that the general features of each management regime drive, even under the same type of plants (in this case, oil palm), the structuring of distinct microbial groups.

Moreover, the dbRDA results (Figure 6) likewise split the data set into two sample groups based in CA and AFS. It was further observed that the microbial community structure of bacteria and fungi was shaped by about 7 major variables (biotic and abiotic) ($p < 0.05$). With 83.2% of the data variation explained (first two axes) for bacteria (Figure 6a) and 63.4% for fungi (Figure 6b), these variables include pH, Mg^{2+}, and soil moisture, as well as arylsulfatase and β-glucosidase activity, and the C contents present into POM and MAOM pools of the SOM (except Mg^{2+} for bacteria and C-MAOM for the fungal community). All significant variables were better related to AFS than to CA.

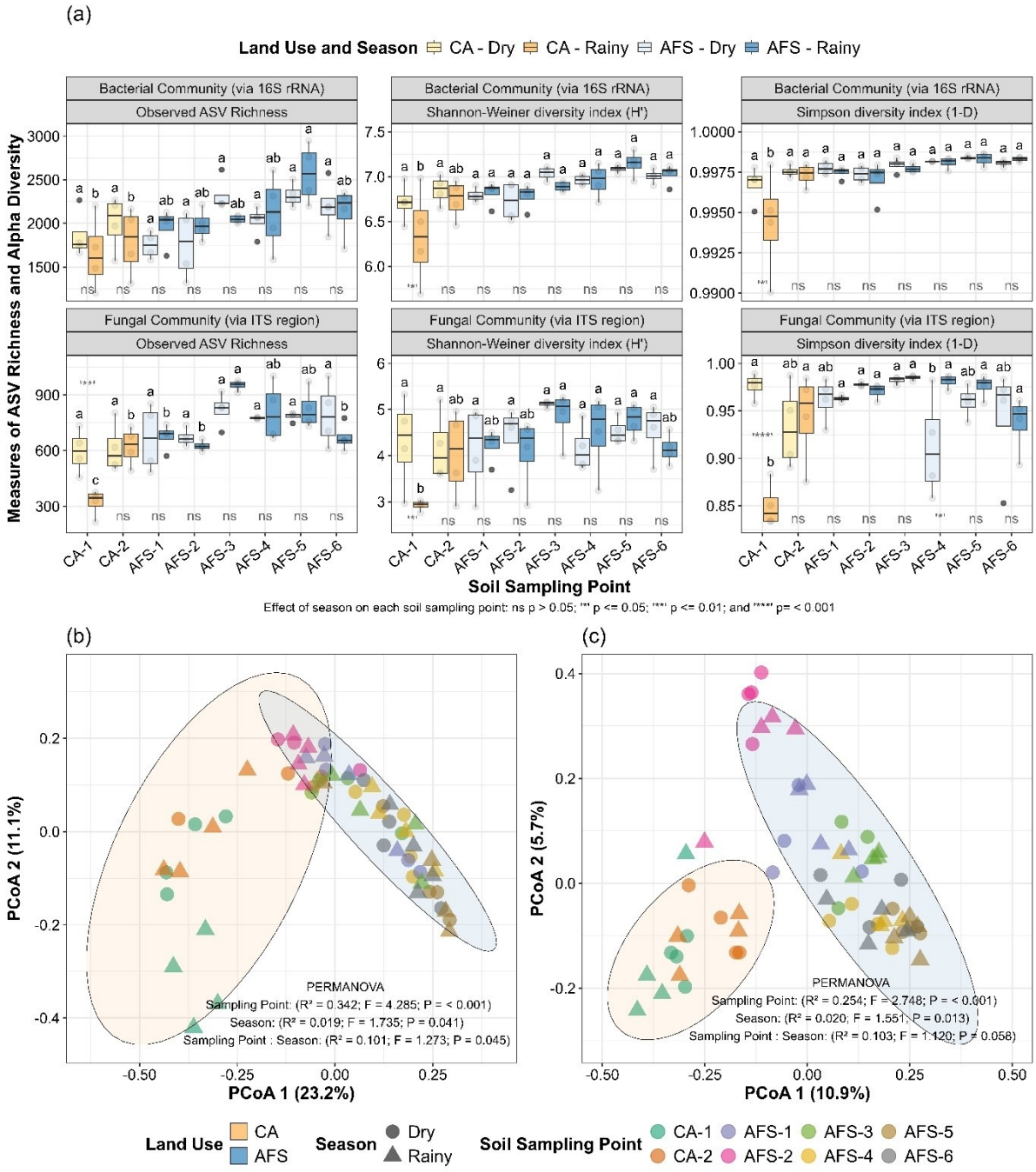

Figure 5. Measurements of observed ASV richness and microbial diversity (bacteria and fungi) (a) and community structure of bacteria (b) and fungi (c) revealed through Principal coordinate analysis (PCoA) according to the 8 sampling points (2 in CA and 6 in AFS) and sampling season at 0-10 cm depth. Ellipses symbolize the dispersion of samples within each land use at 95% confidence. Mean values followed by the same letter do not differ significantly among sampling points ($p \leq 0.05$). Significance codes for differences within sampling points due to sampling season: ns $p > 0.05$, '*' $p \leq 0.05$, '**' $p \leq 0.01$, and '***' $p \leq 0.001$. Values are reported as mean ± standard error (n = 4). CA: conventional agriculture; and AFS: agroforestry system. The other abbreviations are explained in the "Methods section", Table 1.

Finally, specific biological, chemical, and physical variables affected the bacterial and fungi community profile (5 most abundant phyla), as related for the partial

Mantel tests (Figure 7). Predominantly, pH, Ca^{2+}, and Mg^{2+}, followed by H+Al, C-MAOM, and soil sand content were the attributes that were most significantly associated ($p < 0.05$) with the bacterial phyla Acidobacteriota, Actinobacteriota and Proteobacteria, along with Planctomycetota and Chloroflexi in our study sites (Figure 7a). Variations in the fungal phyla Rozellomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, and Ascomycota were also correlated with pH, Mg^{2+}, and Ca^{2+} (except for Rozellomycota), but to a lesser strongly than for bacteria (Mantel's r ~ 0.3-0.6). In addition, H+Al, C-MAOM, and sand content played an important role in modulating fungal taxonomy (Figure 7b). Rozellomycota was the group least linked to the measured variables (only pH and Mg^{2+})

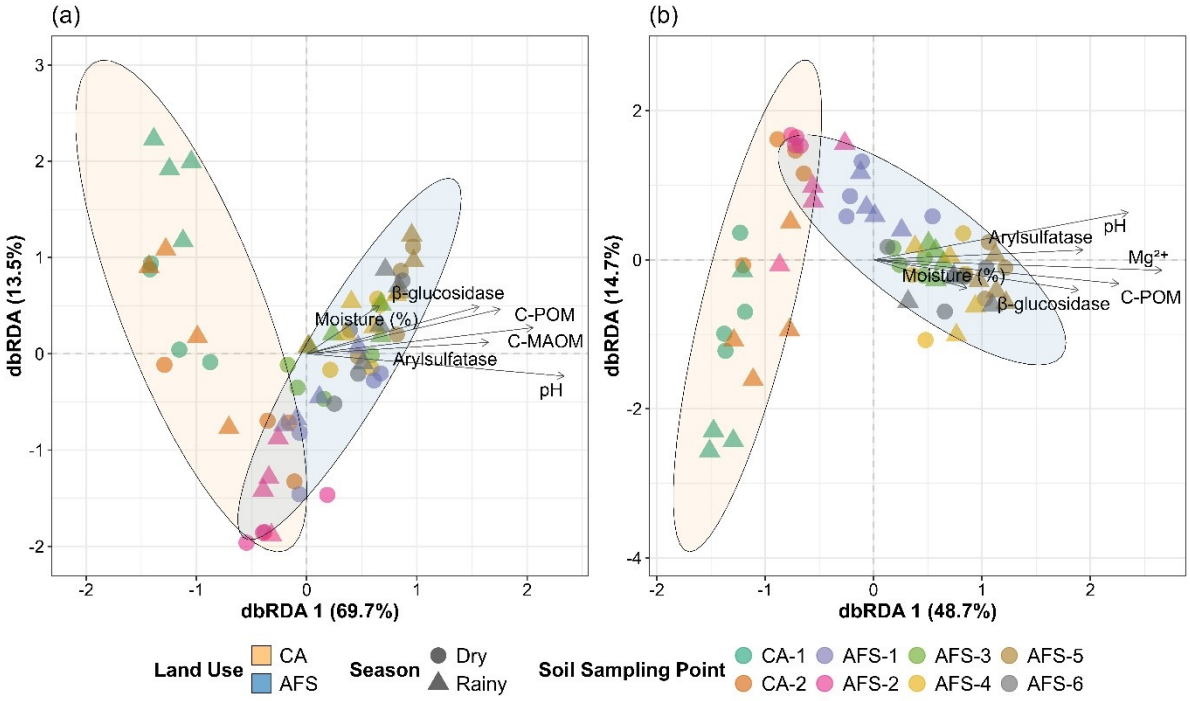

Figure 6. Distance-based redundancy analysis (dbRDA) of the bacterial (a) and fungal (b) community and the biological, chemical and physical attributes of the soil. Vectors represent the measured variables, which were forward selected throughout the dataset (only variables with $p \leq 0.05$ are shown). CA: conventional agriculture; and AFS: agroforestry system. The other abbreviations are explained in the "Methods section", Table 1. C-POM: carbon content present in the > 53 µm physical fraction of soil organic matter (SOM); C-MAOM: carbon content present in the < 53 µm physical fraction of SOM; pH: soil hydrogen potential; $H+Al^{3+}$: potential acidity in the soil; Ca^{2+}: calcium content in the soil; Mg^{2+}: magnesium content in the soil; K^{+}: potassium content in the soil; Moisture (%): soil moisture.

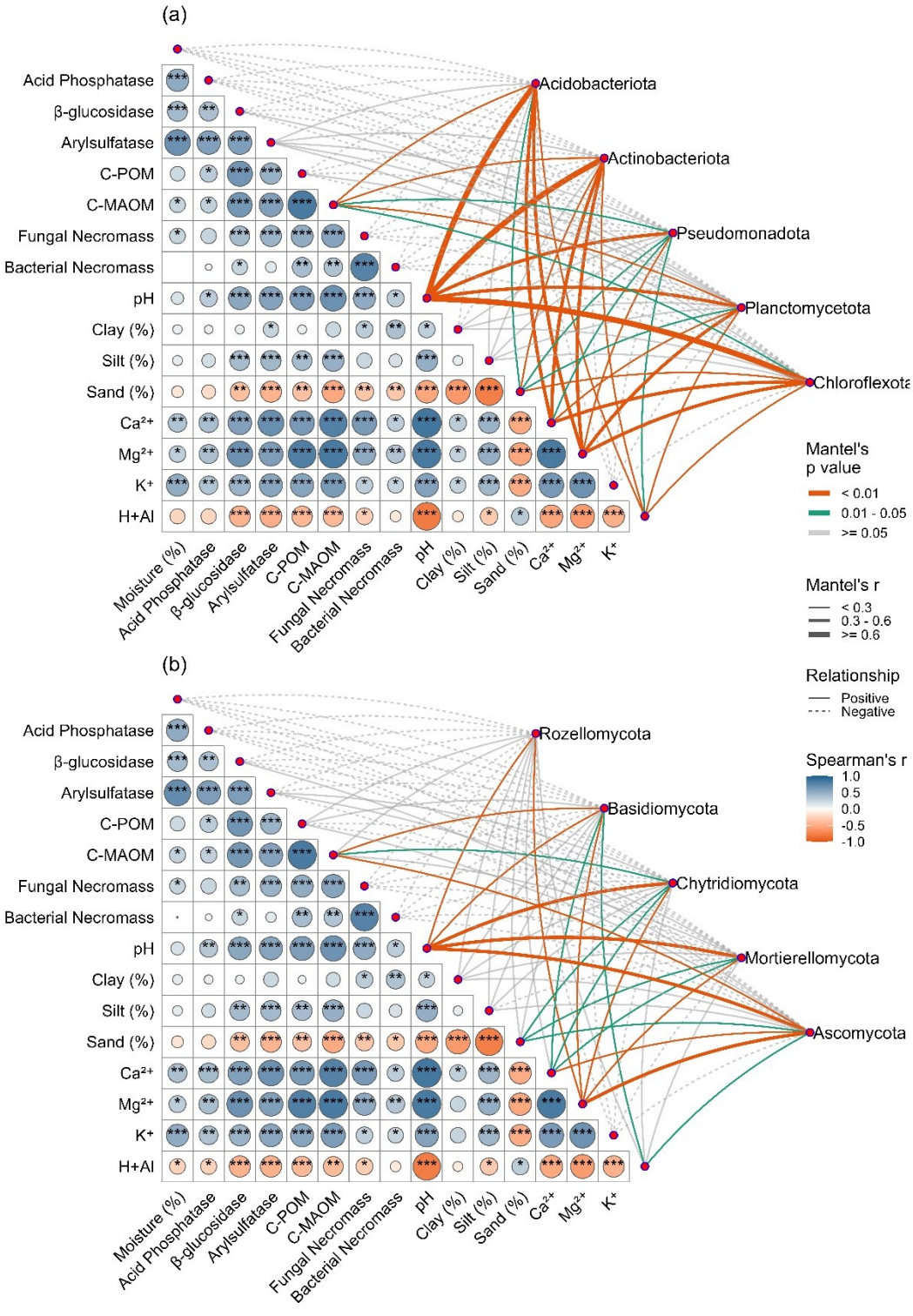

Figure 7. Partial Mantel tests between measured variables (physical, chemical and biological) and the communities of the dominant phyla (5 most abundant) of bacteria (a) and fungi (b). The heatmap shows the pairwise correlations between environmental variables. The lines denote mantel test results, with the line width represents Mantel's r statistic, the type of line representing the relationship (positive or negative), and the color represents Pearson's correlation coefficient. Moisture (%): soil moisture; C-POM: carbon content present in the > 53 µm physical fraction of soil organic matter (SOM); C-MAOM: carbon content present in the < 53 µm physical fraction of SOM; pH: soil hydrogen potential; $H+Al^{3+}$: potential acidity in the soil; Ca^{2+}: calcium content in the soil; Mg^{2+}: magnesium content in the soil; K^{+}: potassium content in the soil.

3.5. Exploring the relationships between microbial necromass and soil biotic and abiotic factors

Bacterial necromass (BN) and fungal necromass (FN) showed a significant and positive correlation with the physical-chemical attributes of the soil and also with the microbial traits in both the dry and rainy seasons (Figure S3). Arylsulfatase activity, C-POM, and C-MAOM, alongside pH, Ca^{2+}, Mg^{2+}, K^{+}, FN, and clay silt contents were collectively (joint assessment of the two seasons evaluated) the most important indicators correlated with the BN levels. On the other hand, FN was strongly associated with the activity of soil enzymes (β-glucosidase, arylsulfatase and acid phosphatase), the Simpson bacterial diversity and Shannon fungal diversity indices, as well as C-POM, C-MAOM, BN, pH, Ca^{2+}, and Mg^{2+}. Furthermore, clay and silt content and soil moisture were also linked to FN levels, while sand content and H+Al exhibited a significant and negative correlation with this variable (sand content also showed negative interaction with BN).

Besides, the results of boosted regression tree analysis (BRT) indicated that clay content and C-POM, followed by Simpson's and Shannon fungal diversity indices, and β-glucosidase activity were the five biggest factors in explaining BN variation across our study sites (CA and AFS) at 0-10 cm depth (Figure 8a). Meanwhile, Ca^{2+}, clay content, and C-POM dominated the FN variation, jointly with C-MAOM and arylsulfatase activity based on the fitted model (Figure 8b). The relative importance of biological, chemical, and physical attribute in reporting BN variation were 58.4%, 8.72%, and 32.88% (R^2 = 0.81), respectively. For the FN, the accumulated biological attributes (55.2%) also played a key role in explaining the variation of this indicator, trailed by chemical attributes (24.96%) and physical attributes (19.84%) (R^2 = 0.74

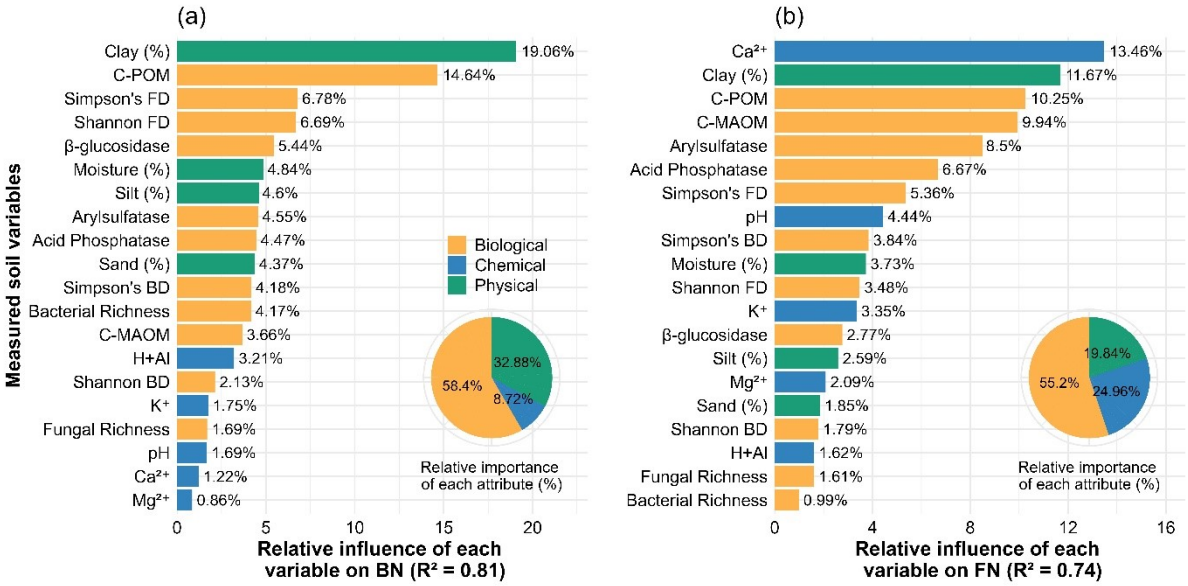

Figure 8. Relative influence of the measured variables on explaining the variation in Bacterial Necromass (BN) (a) and Fungal Necromass (b) carbon estimated via boosted regression tree analysis (learning rate = 0.001). Bars with the same fill color indicate that the variables belong to the same type of soil attribute, including biological, chemical, and physical. C-POM: carbon content present in the > 53 µm physical fraction of soil organic matter (SOM); C-MAOM: carbon content present in the < 53 µm physical fraction of SOM; pH: soil hydrogen potential; $H+Al^{3+}$: potential acidity in the soil; Simpson's BD: Simpson's bacterial diversity index; Simpson's FD: Simpson's fungal diversity index; Ca^{2+}: calcium content in the soil; Shannon BD: Shannon's bacterial diversity index; Mg^{2+}: magnesium content in the soil; Shannon FD: Shannon's fungal diversity index; K^{+}: potassium content in the soil.

4. DISCUSSION

4.1. Intensification of land use through agroforestry increases soil microbial activity, but the effect can be seasonally defined

Overall, the conversion of oil palm plantations from CA to AFS enhanced the potential activity of the extracellular enzymes β-glucosidase and arylsulfatase in the soil depths evaluated (0-10 cm, 10-20 and 20-30 cm, with few exceptions). These findings reinforce the adoption of these two enzymes as early and sensitive indicators to assess the effects of land use changes on microbial activity in Brazilian croplands (Carneiro et al., 2024; Lopes et al., 2018, 2013). However, these shifts were not homogeneous along the sampling points (row and/or inter-row) for both land uses, with the above-ground plant biomass and the sampling season being decisive in the performance of these enzymes. These results were associated, especially in the soil upper layer (0-10 cm), with greater availability and diversification of labile C sources from plants present in the AFS (mainly in SID, AFS-3 to AFS-6). This relationship between C and microbial activity converges with the high levels of C-POM present in agroforestry (Figure 4a) and is consistent with those reported by Lopes et al. (2013).

Furthermore, these enzyme activity results may be related to the influence of the exudation profile of each plant and the more enriched litter leaching resulting from planting multiple species on the soil biochemical component in AFS than in CA (Mendes et al., 2013; Sokol et al., 2019). Interestingly, we realized that at depths of 10-20 cm and 20-30 cm, there was a significant reduction in β-glucosidase activity in the rainy period compared to the dry. Although we are unaware of evidence of a direct relationship between these factors, we argue that this phenomenon may have occurred due to excess water in the soil, since the activity of some enzymes are positively related to the availability of oxygen (Li et al., 2021). Based on Rainfall Estimates from Rain Gauge and Satellite Observation (CHIRPS) data (Funk et al., 2014), there was a variation of ~250 mm in precipitation index between dry and rainy season collections at our sites (Table S3).

This high rainfall might eventually have saturated most of the pores with water (even under sandy soil), altering the soil redox potential toward anaerobiosis via oxygen decrease (process described by Hartmann & Six (2022)) and then decreasing the abundance of β-glucosidase-producing microbial groups; or even had β-

glucosidase levels leached into the soil due to high rainfall volumes (Fetzer et al., 2021; Wirth et al., 2008). Tonon-Debiasi et al. (2024) also reported a decrease in cellulase activity in a Ferralsol because of diminished air-filled pore space in the soil; however, the indicated cause was an increase in soil compaction degree rather than an increase in water content. Finally, the potential acid phosphatase activity was not a good indicator to distinguish CA from AFS at 0–10 cm depth, as this was strongly shaped by season. During the dry season, for instance, we did not identify differences between the land uses studied, but this may have taken place not necessarily due to the lack of efficiency of this indicator, but to the abiotic conditions of the highly weathered soils of the Amazon.

These Amazon soils are naturally poor in nutrients, acidic and with high levels of metal oxides (Fe, Al), which favors the adsorption of inorganic P (Gama-Rodrigues et al., 2014; Moran et al., 2000; Sombroek, 1984). As a consequence, greater microbial action is required to deal with the scarcity of this element in the soil conditions in both CA and AFS. This might suggest a potential direction of microbial strategy towards resource acquisition traits at the expense of other strategies (sensu Malik et al., 2020). Another possibility is that microbial groups producing acid phosphatase were in a dormant condition due to reduced soil moisture during the dry season. This would explain the increased enzyme activity in the rainy season at the same depth (0-10 cm), as "hot moments" emerged in the soil (Kuzyakov & Blagodatskaya, 2015). Likewise, this could account for the differences observed in the other soil layers (10-20 and 20-30 cm) during the dry season itself, where soil moisture is typically more stable and acid phosphatase activity differed between land uses, with AFS generally prevailing over CA.

4.2. Agroforestry promotes microbial necromass pools, but the contribution to SOC is equal or greater in oil palm monoculture

4.2.1. Changes in microbial necromass contents of C

Our results reveal that oil palm-based agroforestry promoted fungal necromass (FN) and total necromass (TMN) pools. Conversely, it had little or no influence on bacterial necromass (BN) contents compared to oil palm monoculture (CA). In any case, the magnitude of accumulation on the three measured variables

was also dependent on the vegetation cover within each land use (planting row and inter-row) and the seasonality across the evaluated depths (0-10, 10-20, and 20-30 cm). Except for BN, our findings converge with those described by Xu et al. (2023) and Qian et al. (2023), who observed an increase on FN and TMN levels in response to increased plant diversity in forest soils. According to Qian et al. (2023), the preferential adsorption of microbial residues on mineral surfaces enhanced by biotic mineral weathering by plants and the increase in carbon use efficiency (CUE) were the central actors of these results. Xu et al. (2023), in turn, linked the improvement in necromass contents to the increase in microbial biomass C (a proxy for necromass) and changes in soil beta diversity.

We acknowledge that CUE was not measured in our experimental design, which prevents us from drawing direct associations based on this physiological index. However, higher pH values in AFS compared to CA were verified (Table S2), which may benefit a higher CUE by alleviating acid repression of microbial activity (Malik et al., 2018). Similarly, soil microbial activity measured by extracellular enzymes (a proxy for biomass C) (Carneiro et al., 2024; Lopes et al., 2018, 2013) was generally higher in AFS and therefore may have aided SOM turnover and subsequent accumulation of biomass then necromass. Qian et al. (2023) claimed that more diversified systems, as applicable to our study, induce competition for resources among plants, leading to an expansion of root structures. This expansion increases root biomass and length, alters the root exudation profile, and results in a greater input of low molecular weight organic acids (Qian et al., 2023). These acids may act as "mining agents" on soil surfaces, promoting the release of minerals that can bind to microbial residues and thus enhance soil necromass pool via organo-mineral associations (Buckeridge et al., 2022; Keiluweit et al., 2015).

That rationality is relevant and ecologically useful to support our results for FN and TMC as well, since the agricultural practices employed in CA and AFS are long-term. It was worthy to mention that the better physical quality metrics at the AFS site, namely soil bulk density and aggregation (results previously reported by Oliveira et al. (2022a) and Gomes et al. (2024)), could also have contributed to achieving the higher levels of FN and TMC. The main mechanism for this contribution would be physical protection via occlusion (burial) of C-based microbial residues within the aggregates in AFS (Carvalho et al., 2023). This view is encouraged by the results of Zhao et al. (2023), who found great amount of fungal necromass C in macroaggregates (>250

μm) induced by root traits under intercropping. That said, we suggest a complementary inter-relationship among the biological, chemical, physical pathways showed here as a reliable explanation for the increase in FN and also TMC levels in our study, since the volume of FN represented the majority of microbial C pool in both CA and AFS.

Regarding BN, we propose specific reasons for the low contents compared to FN, which involve the consensus on the larger living fungal biomass than bacterial biomass detected in soils worldwide, and the acidic condition observed at our sites (pH ≤ 4.9) (B. Wang et al., 2021; Yang et al., 2022). Together, these factors may have augmented the fungal biomass and thus resulting in less BN being continuously accumulated than FN. On the other hand, the low sensitivity of BN to changes in land use was also reported by Li et al. (2022) and may be related to the faster turnover of bacterial biomass compared to fungal biomass. This greater bacterial turnover could be explained by two main mechanisms: more efficient microbial recycling (use of necromass as a source of C by microbial community before stabilization) (Buckeridge et al., 2020) and/or destabilization of necromass due to microbial mining (Buckeridge et al., 2022) preferentially targeting bacterial necromass.

The higher efficiency of bacterial necromass recycling was suggested by Prommer et al. (2020) as a potential pathway to elucidate the low sensitivity of BN to increased plant biomass. This may occur due to the preference of native soil microbes for bacterial necromass as substrate and was the reason assigned by Li et al. (2022) to the low sensitivity of BN levels in soils under different land use. The rationale is based on the lower C:N ratio of bacterial necromass) (Strickland & Rousk, 2010) and the potential high melanization present in fungal necromass (Maillard et al., 2023). Besides, environmental conditions, such as historical precipitation, have been reported to be a more important factor than the intensity of agricultural practices in regulating necromass recycling rates (Buckeridge et al., 2020). This result provided by Buckeridge et al. (2020) also assist us to understand the changes imposed by seasonality (dry and rainy) on microbial necromass levels at our sites along the soil depth evaluated, since the Wei et al. (2023) explanations for similar pattern were inconclusive. Hence, these findings collectively endorse our argument that more efficient recycling of BN, associated with the specific environmental conditions of our sites and which can be decoupled from management, may have shaped the content of this SOC component.

Additionally, the BN could be more easily lost (destabilized) through microbial mining due to the preference for bacterial substrates in soil connected with the increased intensity of this mining at sampling points located in AFS than in CA. The theoretical basis for the preferential use might be the same as discussed earlier, while the increased intensity in AFS could have as background the increased microbial activity observed in our agroforestry (Table 2) and the differences in microbial community structure across different land uses (Figure 5b, c) - commonly linked to the highest inputs of C substrates under agroforestry (Visscher et al., 2024). Thereby, it is logical to reason that these agroforestry soils, which usually exhibit enhanced microbial traits compared to conventional plantations, also host microbial communities with more diverse decomposition skills and thus presumably greater microbial C mining on mineral surfaces. This would reinforce the crucial role of ecological metrics of soil microbes in C necromass turnover (Buckeridge et al., 2020; Maillard et al., 2023; Xu et al., 2023). Furthermore, it would provide insights into a possible inter-relationship between substrate preference (i.e., necromass chemistry) (Maillard et al., 2023) and different levels of microbial mining based on land use intensity. This is a promising hypothesis to be tested in future research. For this reason, new studies to test this theoretical framework are encouraged and stable isotope tracking appears to be a useful tool for this purpose.

4.2.2. Necromass pool as a proportion of SOC

Lastly, we found that the contribution of microbial necromass (BN, FN, and TMN) to SOC was generally lower in AFS ($p \leq 0.05$) or did not show statistical differences between oil palm monoculture sampling points compared to AFS (Figure 3). The contribution of TMN, for instance, accounted for ~28% (at maximum) in AFS-2 at 10–20 cm depth. This indicates that changes in microbial necromass were directly proportional to changes in SOC content across our study sites. Therefore, there was a kind of “buffering effect” in necromass contribution to SOC, suggesting a weak effect of land use conversion on this relationship. These results were unexpected, since the progressive increase in our C indicators under agroforestry (Figure 3 and Figure 4) could also suggest an increase in the necromass:SOC ratio in favor of AFS, but this remarkably did not happen. Although intriguing, these findings are partially in line with

those of Jia et al. (2022) and Qian et al. (2023), as well as the recent publication by Li et al. (2024).

Concurrently, both Angst et al. (2024) and Li et al. (2024) also observed, as we did, a relative stability and sometimes an increase in the necromass pool as a proportion of SOC along the vertical soil profile (0-10, 10-20 and 20-30 cm). The reason given by these authors for the low sensitivity or lack of change in the necromass:SOC ratio was unclear (deterministic) and partially attributed to the similar soil conditions among the soil management systems evaluated by them. This includes very similar precipitation regime, temperature and soil parent material. Another cause pointed out was the influence generated by specific microbial traits of each soil, such as the dominance of certain microbial groups at the expense of others, in soil organo-mineral interactions, resulting in unchanged contribution of necromass to the SOC (Li et al., 2024). In our study, some of the microbial community traits were indeed distinct between the sites. The notable divergences in the structure of bacterial and fungal communities between sampling points distributed in AFS and those located in CA are a good example.

However, the results for the necromass:SOC ratio between the two land uses studied appear to be decoupled from community structure and also from the divergent C contents between CA and AFS. Thus, the predominant effect of edaphic conditions in our study in relation to agricultural practices and pre-existing variations in soil depth in determining the contribution of necromass to SOC is plausible (Angst et al., 2024; Jia et al., 2022; Li et al., 2024; Qian et al., 2023). Even though soil microbes and agricultural practices are important drivers of soil C fluxes, edaphic factors likely also shaped the mechanisms that underlie organo-mineral interactions. One measure that may corroborate this point is the FN:FB ratio (Figure S4), which was little changed across the sampling points in CA and AFS, as well as in the evaluated soil layers (with exceptions linked to variations in the necromass:SOC ratio). This may impose uniform limits on the contribution of microbial necromass to the SOC pool, even under more or less diversified agricultural crops, as indicated by Qian et al. (2023) and supported by the observed data. Regardless, a factor unrelated to the experimental design and/or the methods employed in our study to elucidate this phenomenon should not be ruled out, since mechanistic causes have not yet been revealed and the results available in the literature are highly unclear.

4.3. Agroforestry is more efficient in accumulating C as POM and MAOM pools than oil palm monoculture

The significant increase in C contents in the physical pools of SOM (i.e., POM and MAOM) after the conversion of traditional oil palm monocultures (CA) to AFS provides important implications for the C dynamics under these crops. Our data verify that multi-species plantation maximized total SOC in the Amazon, as previously reported by Carvalho et al. (2014). In addition, they offer useful insights into the quality of the accumulated carbon (origin, lability, and persistence) (Lavallee et al., 2020). Moreover, they indicate the key role of the type of land cover, that is, the plants that will be employed in agroforestry systems aiming at C sequestration in the soil as a nature-based strategy. This was demonstrated by the heterogeneous distribution of C along the sampling points located within CA and AFS and in all soil layers evaluated (0-10, 10-20, and 20-30 cm). For AFS specifically, the larger C contents in POM and MAOM were mostly found in the strip under integration and diversification (SID, i.e., AFS-3 to AFS-6), composed of regional Amazonian crops, such as cocoa, açaí and trees.

On the other hand, these findings also demonstrate that oil palm, even under agroforestry system, is not efficient to increase soil C pools by itself (as verified by the little or no differences among CA-1, CA-2, AFS-1, and AFS-2). These results partially converge with studies previously carried out in tropical environments and which also employed experimental designs focused on the dynamics of the physical reservoirs of SOM (Locatelli et al., 2022; Oliveira et al., 2022b; Pimentel et al., 2024). The increases in C-POM were generally related to the greater input of low-quality organic substrates from plants into the soil (i.e., high C:N or lignin:N ratio), although Angst et al. (2024) also pointed out microbial necromass as an important contributor to the C-POM pool. Due to their chemical structure (highly heterogeneous), these substrates are considered more recalcitrant and are less efficiently assimilated by native microbial biomass, so they remain in the soil as incompletely decomposed organic particles for longer and stimulate the formation of POM (Cotrufo et al., 2013).

The theoretical framework outlined above is drawn from the Microbial Efficiency Matrix Stabilization (MEMS) and supports the data observed in our study, given that the largest contribution of organic matter (quantitative and qualitative) in AFS is not only composed by labile substrates, but also of recalcitrant ones that probably

improved C-POM compared to CA. This theory also supports the variations in C-POM between sampling points within AFS, since oil palm, cocoa and açaí contribute different rates of organic matter above and below ground, with C:N ratios also distinct (Brasil et al., 2020). The concentrations of C-POM in AFS were likely favored by no-tillage and improved soil conditions (moisture, temperature, and aggregation) typical of agroforestry systems compared to homogeneous crops (Nair, 2017; Visscher et al., 2024). These improvements can reduce the turnover rates of the particulate C pool, increasing its accumulation and residence time (persistence) in the soil, as POM is “uncomplexed” and highly sensitive to environmental and management stressors compared to MAOM.

Additionally, the MEMS theory helps us understand the depletion of C-POM observed along the soil layers, as the effects of organic matter inputs to the soil are usually confined to the surface (0-10 cm) and reduced down along the vertical soil profile. As an example, we have the absence of statistical differences in C-POM among all sampling points in both CA and AFS at the depth of 20-30 cm during the rainy season campaign (Figure 4a). From an integrated perspective, our findings demonstrate the quantitative increase in C-POM levels relative to SOC after adopting AFS instead of CA, but also bring functional implications for SOM dynamics. POM is an important nutrient reservoir in soil (for both microbes and plants) and is commonly used as an indicator of potential C cycling. Given that this more labile fraction is not subject to saturation, management strategies that target POM accumulation, such as agroforestry, offer a realistic opportunity for C sequestration in Amazonian oil palm fields (Angst et al., 2023; Pimentel et al., 2024).

Regarding the C-MAOM contents, patterns similar to C-POM were verified. This increase was highly desirable, since C-MAOM is accounted more stable and has a residence time estimated in centuries (Cotrufo et al., 2019; Lavallee et al., 2020). The augmentation compared to CA was up to 200% (depending on the sampling point and season) and reflects the efficiency of the agroforestry system to promote the soil a persistent C sink. This greater persistence of C-MAOM is due to the protection of C accumulated in the soil by chemical (association with mineral surfaces) or physical (occlusion in aggregates) pathways and can also be supported by MEMS theory. Unlike C-POM, Cotrufo et al. (2013) posit that C-MAOM is mainly composed by labile compounds from plants (leaf leachate and/or rhizodeposition) and that they are easily sorb to mineral surfaces (“direct sorption”) or assimilated by the microbial community

through the “microbial carbon pump” (MCP) (Liang et al., 2017). After the microbial life and death cycles, part of the C retained in the biomass becomes available as microbial necromass and is then associated with soil mineral surfaces (Buckeridge et al., 2022).

The dominance and relative contribution rate of C-MAOM in the SOC pool (average of ~90%) in relation to C-POM (average of ~10%) is compatible with most soils (Curtin et al., 2019). This is justified by the mechanisms of formation, accumulation and residence time of each physical fraction, as discussed above. Besides, the evidence provided in our study demonstrates a “win-win combination" between these two SOM pools under AFS, that is, a proportional increase in both POM and MAOM concentrations. This suggests the skill of long-term oil palm agroforestry to provide both more recalcitrant and more labile compounds, thus stimulating the formation of different forms of soil C. The significant and positive correlation between MAOM and POM contents exposed by Spearman's matrix (Figure S3) also encourages this relationship and may further suggest a specific pathway for MAOM formation at our sites, with POM as a possible precursor via direct sorption of dissolved organic matter or biotic transformation of POM (Angst et al., 2023). Finally, the efficiency of multispecies planting in AFS in turning the soil into a C sink, compared to CA, is noteworthy. Even under naturally similar physicochemical properties between the investigated land uses, such as pH and $H+Al^{3+}$ levels (potential acidity), as well as clay content (Table S3) (often considered limiting factors for carbon accumulation in soils) (Wang & Kuzyakov, 2024), agricultural management stood out and proved to be a key factor in soil carbon levels.

4.4. Agroforestry and oil palm monoculture exhibit divergent microbial communities

4.4.1. Taxonomic composition of microbial communities

The analysis of the taxonomic composition of the bacterial community revealed a higher relative abundance of the phyla Proteobacteria, Actinobacteriota, and Acidobacteriota, as well as Planctomycetota and Chloroflexi (in this order) at the sampling points in CA and AFS. This profile was relatively consistent across the sampling points in our sites and even under variations in the seasonality of precipitation (dry and rainy). However, the predominance and relative stability in the abundance of

these phyla over other less abundant ones does not imply a homogeneity of the bacterial communities nor a low response of the composition to the features of our sites (CA and AFS), since deeper phylogenetic analyses (such as at the family and genus level) can reveal significant taxonomic differences. In fact, the bacterial profile observed is in agreement with the recent study of molecular characterization of microorganisms in tropical soils carried out by Silva et al. (2024).

In contrast to bacteria, the composition of the fungal community was quite sensitive to the agricultural practices that characterize CA and AFS. The main groups identified were Ascomycota (which dominated the community), Basidiomycota and Chytridiomycota, followed by Mortierellomycota and Rozellomycota. A portion of ASVs (up to ~20%, depending on the soil sampling point) did not have their taxonomy assigned due to a lack of match with the pre-defined 99% similarity. These unmatched sequences may have occurred due to the absence of sequences obtained from the reference dataset (UNITE v.10) or problems related to the sequencing technique itself (like DNA quality and PCR bias, among others). In any case, the predominance of Ascomycota, Basidiomycota and this non-classification rate close to 20% corroborate the results of Egidi et al. (2019), who studied the distribution patterns of fungi in 235 soils from around the world.

4.4.2. Measures of community richness, diversity and structure

The observed richness of ASVs (to bacteria and fungi) was higher at sampling points in AFS than in CA, specifically in the rainy season. However, some exceptions were identified for fungal richness, which did not differ between points that contained oil palm (CA-1, CA-2, AFS-1, and AFS-2). This indicates the potential of agroforestry to provide specific niches for the establishment of ASVs (sometimes unique) (Figure S5) due to biotic and/or abiotic factors (Barros et al., 2021). These factors, however, have their effects minimized during the dry season and are affected by above-ground biomass. Regarding microbial diversity, we identified a decoupling between alpha and beta metrics for both bacteria and fungi in AFS and CA. While alpha diversity (Shannon and Simpson indices) of bacteria and fungi showed little to no divergence between our sites, beta diversity clearly exhibited two sample groups based on CA and AFS.

These results to alpha diversity align with previous studies carried out in the Amazon (Mendes et al., 2015a; 2015b; Navarrete et al., 2015). Nevertheless, our

findings do not imply ecological homogeneity of microbial communities (bacteria and fungi), as our data are taxonomic and not functional. Pedrinho et al. (2023), for instance, indicated that abiotic stress conditions frequently detected in pastures increase taxonomic diversity in Amazonian soils. Thus, we can advocate that the absence of significant differences in alpha diversity (with minor exceptions) may be related not necessarily to the low diversity present in AFS, but to the high diversity in CA. This increase in diversity typically also promotes functional diversity, leading to functional redundancy, an important microbial strategy to overcome environmental disturbance, and thus maintain key microbially-mediated processes in the soil (Mendes et al., 2015a; Pedrinho et al., 2023).

Conversely, significant divergences were observed for beta diversity of bacteria and fungi at our sites. This reveals the critical role of agricultural practices in governing soil microbial diversity and, therefore, the ecological arrangements and the functions performed by microbial communities (Andreote & Silva, 2017). When evaluating the dynamics of microbial communities under AFS in the Amazon, Leite et al. (2023) found that agroforests create specific habitat conditions for bacteria and archaea. These conditions differed from those of natural rainforest soils, and our results now indicate that they also differ from soils under monoculture in biological, chemical and physical terms (as reported in the previous sections 4.1, 4.2, and 4.3). This highlights agroforestry as an “intermediate” land use strategy between monocultures and forests, with real potential to mitigate the effects of conventional agricultural practices on soil microbial diversity.

Typical of many soil microbial investigations (Buckeridge et al., 2020; Mendes et al., 2015a; Navarrete et al., 2013), pH was the most important chemical property to explain variation in community structure for both bacteria and fungi, trailed by Mg2+ levels for fungi. This can be justified by the fact that pH is a major variable in soil, that is, capable of determining other factors, including Mg^{2+} (Navarrete et al., 2013; Wang & Kuzyakov, 2024). As a result, subtle variations in pH can trigger additional changes in soil (such as nutrient availability) and thus select specific microbial communities under environmental gradients. In our study, pH and Mg^{2+} were correlated with the microbial community in AFS (Figure 6). This is in agreement with our expectations, given that pH was higher in agroforestry compared to oil palm monoculture and has a positive relationship with Mg^{2+}.

These chemical interactions also pave the way for understanding why pH was the chemical variable that most strongly affected the top 5 bacterial and fungal phyla in the soil alongside with Ca^{2+}, Mg^{2+}, and H+Al (except for fungi) (Figure 7a, b). In parallel, physical and biological variables also associated with the structure and composition of microbial communities. Soil moisture, arylsulfatase and β-glucosidase activity, as well as the physical C pools of SOM played a significant role in explaining the variations of our dbRDA (Figure 6). These variables were, in their entirety, related with the sampling points in the AFS. Such findings converge with those of Araujo et al. (2021) and Navarrete et al. (2015), who pointed out soil moisture and C levels as conditioners and energy sources for microbial life (respectively), capable of defining community traits. However, soil moisture did not prove to be a core factor in the composition of the major bacterial and fungal phyla, suggesting its more prominent role in determining beta diversity rather than taxonomic profile.

Ultimately, sand content was a physical attribute positively correlated with all bacterial phyla, but to a lesser extent with fungal ones. This reveals a tolerance mechanism of this last group, since soil texture was ranked by Xia et al. (2020), after pH, as the main factor in the modulation of soil microbial communities. At the same time, the lower influence of H+Al on the formation of most fungal phyla is due to the physiological adaptations of these microbes compared to bacteria for more acidic conditions (Wang & Kuzyakov, 2024). The significant effect of β-glucosidase and arylsulfatase activity, in turn, can be accounted to the influence of C pools on the variability of AFS communities. This rationale is based on the fact that these two enzymes are closely linked to soil C turnover (although via indirect pathways, in the case of arylsulfatase) (Carneiro et al., 2024; Klose et al., 2015). Thus, it could be assumed that the microbes present in the AFS are more dependent on the continuous supply of C sources; this also implies, on the other hand, that the microbes in CA may be more resilient to variations in this element.

4.5. Microbial necromass C is predicted by a combination among biological, physical and chemical soil attributes

We C-POM and C-MAOM played a critical role in the necromass pool at our sites, since microbial residues are part of the SOC. Given that BN and FN were not measured within the physical pools of SOM, we intentionally included C-POM and C-

MAOM in the BRT model. With this approach we indicate that BN may have potentially contributed more to C-POM, while FN played an important role in both C-POM and C-MAOM. The influence of Simpson and Shannon's fungal diversity, in turn, is coherent because necromass levels are mediated by the proportion of soil fungi to bacteria and, consequently, by the FN:BN ratio (Liang et al., 2019; Strickland & Rousk, 2010). Thus, it can be assumed that fungal diversity (related to fungal abundance) (Melo, 2008) could have affected ecological processes that mediate the fungi:bacteria ratio in the soil and then the BN contents.

The activity of β-glucosidase for BN and arylsulfatase for FN is related to the role of these enzymes in soil C turnover, as explained in section 4.4.2. As a rule, β-glucosidase acts in the breakdown of cellulose and the conversion of cellobiose into glucose molecules and, therefore, has a direct role in the decomposition of SOM (Carneiro et al., 2024). Arylsulfatase, on the other hand, is coupled to the hydrolysis of sulfate esters and thus to the release of sulfate anions into the soil (Klose et al., 2015). However, SOM is the main source of S for microbes and plants (Pinto & Nahas, 2002; Vitti et al., 2015), so it is rational to suppose that arylsulfatase activity can also influence C fluxes in the soil and, by extension, microbial necromass. Another pathway would be β-glucosidase and arylsulfatase as parts of the BN and FN pool itself. This speculation would be encouraged by the findings of Rempfert et al. (2024), who indicated microbial metabolites, that is, microbial residues (an expanded view of necromass) (Whalen et al., 2022) as an additional contributor to soil C accumulation.

Moreover, the influence of Ca^{2+} on FN content may reveal the potential role of this element in supporting necromass accumulation via mineral protection. Although Amazonian soils typically host high levels of metal oxides (important SOM complexing agents in acidic conditions) (Wang & Kuzyakov, 2024), Hu et al. (2022) reported a complementary effect between iron oxides and exchangeable Ca^{2+} in promoting microbial necromass in forest soils. The positive effect would be related to the adsorption of chemical functional groups from the necromass (such as -OH and -COOH) to Ca^{2+} on mineral surfaces through cationic bridges (Qian et al., 2023). Finally, the importance of clay contents in predicting both bacterial and fungal necromass met our expectations, considering the importance of soil minerals in C accumulation due to organomineral associations (Lavallee et al., 2020). However, the effect size was shaped by the type of necromass (bacterial or fungal), demonstrating a plausible

relationship between the chemical composition of the necromass and sorption rates to soil mineral surfaces, as raised by us in the previous sections.

.

5. CONCLUSION

Our results showed that the conversion of traditional oil palm monocultures (CA) into long-term oil palm-based agroforestry (AFS) strongly enhance core attributes of C storage and quality. This was largely demonstrated by the promotion of soil microbial activity (as revealed by the activity of the extracellular enzymes β-glucosidase and arylsulfatase) and the significant increases in the C pools assessed (fungal and total microbial necromass, C-POM, C-MAOM, and total SOC). However, it had little or no influence on the bacterial necromass content, suggesting that these residues have specific accumulation mechanisms in the soil. We propose that these mechanisms involve more efficient recycling and microbial mining directed at "post-mortem" bacterial residues. We also realized that the necromass:SOC ratio in AFS was lower and sometimes equal to CA, and occasionally increased with soil depth. These findings are related to the similar soil edaphic conditions at our sites and the little change in the FN:BN ratio in the soil, although we do not rule out an underlying mechanism not yet revealed in the current research.

Furthermore, agroforestry has assembled distinct bacterial and fungal communities compared to oil palm monoculture by biological, chemical and physical attributes of the soil, with beta diversity being the most evident trait of this effect. This highlights the potential of multi-species plantation to mitigate the impacts of conventional agricultural practices on soil microbial diversity and by extension soil functioning in the Amazon. The critical role of aboveground plant diversity and type in C fluxes was also demonstrated. As a consequence, most of the reported findings were shaped by land cover, underline the heterogeneity but also the niche complementarity existing among plants under oil palm-based agroforestry. Lastly, we provide the first insights for Brazilian croplands into a specific, dynamic and worthy of future study stabilization component in tropical environments: the microbial necromass C. Aware of the observational nature of our study, we encourage future approaches based on processes capable of shaping necromass turnover, including to test the hypotheses raised by us in this investigation. Stable isotope tracking techniques associated with functional assays and molecular biology methods, for instance, seem to be useful tools for this purpose.

REFERENCES

Andreote, F. D., & Silva, M. de C. P. e. (2017). Microbial communities associated with plants: learning from nature to apply it in agriculture. *Current Opinion in Microbiology*, *37*, 29–34. https://doi.org/10.1016/j.mib.2017.03.011

Angst, G., Angst, Š., Frouz, J., Jabinski, S., Jílková, V., Kukla, J., Li, M., Meador, T. B., & Angel, R. (2024). Stabilized microbial necromass in soil is more strongly coupled with microbial diversity than the bioavailability of plant inputs. *Soil Biology and Biochemistry*, *190*, 109323. https://doi.org/10.1016/j.soilbio.2024.109323

Angst, G., Mueller, K. E., Castellano, M. J., Vogel, C., Wiesmeier, M., & Mueller, C. W. (2023). Unlocking complex soil systems as carbon sinks: multi-pool management as the key. *Nature Communications*, *14*(1), 2967. https://doi.org/10.1038/s41467-023-38700-5

Appuhn, A., Joergensen, R. G., Raubuch, M., Scheller, E., & Wilke, B. (2004). The automated determination of glucosamine, galactosamine, muramic acid, and mannosamine in soil and root hydrolysates by HPLC. *Journal of Plant Nutrition and Soil Science*, *167*(1), 17–21. https://doi.org/10.1002/jpln.200321302

Araujo, A. S. F., Melo, V. M. M., Pereira, A. P. de A., Lopes, A. C. de A., Rocha, S. M. B., Araujo, F. F., & Mendes, L. W. (2021). Arbuscular mycorrhizal community in soil from different Brazilian Cerrado physiognomies. *Rhizosphere*, *19*, 100375. https://doi.org/10.1016/j.rhisph.2021.100375

Artaxo, P. (2023). Amazon deforestation implications in local/regional climate change. *Proceedings of the National Academy of Sciences*, *120*(50). https://doi.org/10.1073/pnas.2317456120

Bai, Y., & Cotrufo, M. F. (2022). Grassland soil carbon sequestration: Current understanding, challenges, and solutions. *Science*, *377*(6606), 603–608. https://doi.org/10.1126/science.abo2380

Barros, F. M. do R., Fracetto, F. J. C., Lira Junior, M. A., Bertini, S. C. B., & Fracetto, G. G. M. (2021). Spatial and seasonal responses of diazotrophs and ammonium-oxidizing bacteria to legume-based silvopastoral systems. *Applied Soil Ecology*, *158*, 103797. https://doi.org/10.1016/j.apsoil.2020.103797

Beidler, K. V., Phillips, R. P., Andrews, E., Maillard, F., Mushinski, R. M., & Kennedy, P. G. (2020). Substrate quality drives fungal necromass decay and decomposer community structure under contrasting vegetation types. *Journal of Ecology*, *108*(5), 1845–1859. https://doi.org/10.1111/1365-2745.13385

Beillouin, D., Corbeels, M., Demenois, J., Berre, D., Boyer, A., Fallot, A., Feder, F., & Cardinael, R. (2023). A global meta-analysis of soil organic carbon in the Anthropocene. *Nature Communications*, *14*(1), 3700. https://doi.org/10.1038/s41467-023-39338-z

Benami, E., Curran, L. M., Cochrane, M., Venturieri, A., Franco, R., Kneipp, J., & Swartos, A. (2018). Oil palm land conversion in Pará, Brazil, from 2006–2014: evaluating the 2010 Brazilian

Sustainable Palm Oil Production Program. *Environmental Research Letters*, *13*(3), 034037. https://doi.org/10.1088/1748-9326/aaa270

Bossio, D. A., Cook-Patton, S. C., Ellis, P. W., Fargione, J., Sanderman, J., Smith, P., Wood, S., Zomer, R. J., von Unger, M., Emmer, I. M., & Griscom, B. W. (2020). The role of soil carbon in natural climate solutions. *Nature Sustainability*, *3*(5), 391–398. https://doi.org/10.1038/s41893-020-0491-z

Brasil, E. C., Cravo, M. da S., & Viégas, I. de J. M. (2020). *Recomendações de calagem e adubação para o estado do Pará* (2nd ed., Vol. 1). Embrapa. https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1125022/recomendacoes-de-calagem-e-adubacao-para-o-estado-do-para

Buckeridge, K. M., Creamer, C., & Whitaker, J. (2022). Deconstructing the microbial necromass continuum to inform soil carbon sequestration. *Functional Ecology*. https://doi.org/10.1111/1365-2435.14014

Buckeridge, K. M., Mason, K. E., McNamara, N. P., Ostle, N., Puissant, J., Goodall, T., Griffiths, R. I., Stott, A. W., & Whitaker, J. (2020). Environmental and microbial controls on microbial necromass recycling, an important precursor for soil carbon stabilization. *Communications Earth & Environment*, *1*(1), 36. https://doi.org/10.1038/s43247-020-00031-4

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Cambardella, C. A., & Elliott, E. T. (1992). Particulate Soil Organic-Matter Changes across a Grassland Cultivation Sequence. *Soil Science Society of America Journal*, *56*(3), 777–783. https://doi.org/10.2136/sssaj1992.03615995005600030017x

Carneiro, R. G., Figueiredo, C. C. de, Malaquias, J. V., & Mendes, I. C. (2024). A soil health assessment tool for vegetable cropping systems in tropical soils based on beta-glucosidase, arylsulfatase, and soil organic carbon. *Applied Soil Ecology*, *198*, 105394. https://doi.org/10.1016/j.apsoil.2024.105394

Carvalho, W. R. de, Vasconcelos, S. S., Kato, O. R., Capela, C. J. B., & Castellani, D. C. (2014). Short-term changes in the soil carbon stocks of young oil palm-based agroforestry systems in the eastern Amazon. *Agroforestry Systems*, *88*(2), 357–368. https://doi.org/10.1007/s10457-014-9689-2

Carvalho, M. L., Maciel, V. F., Bordonal, R. de O., Carvalho, J. L. N., Ferreira, T. O., Cerri, C. E. P., & Cherubin, M. R. (2023). Stabilization of organic matter in soils: drivers, mechanisms, and analytical tools – a literature review. *Revista Brasileira de Ciência Do Solo*, *47*. https://doi.org/10.36783/18069657rbcs20220130

Chen, X., Chen, H. Y. H., Chen, C., Ma, Z., Searle, E. B., Yu, Z., & Huang, Z. (2020). Effects of plant diversity on soil carbon in diverse ecosystems: a global meta-analysis. *Biological Reviews*, *95*(1), 167–183. https://doi.org/10.1111/brv.12554

Costa, L. R. de J., de Matos, G. S. B., Gomes, M. F., Kato, O. R., Castellani, D. C., Guedes, R. S., & Vasconcelos, S. S. (2023). Soil fertility in oil palm agroforestry systems in the Eastern

Amazon, Brazil. *Agroforestry Systems*, *97*(5), 865–881. https://doi.org/10.1007/s10457-023-00832-4

Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience*, *12*(12), 989–994. https://doi.org/10.1038/s41561-019-0484-6

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. https://doi.org/10.1111/GCB.12113

Creamer, C. A., Foster, A. L., Lawrence, C., McFarland, J., Schulz, M., & Waldrop, M. P. (2019). Mineralogy dictates the initial mechanism of microbial necromass association. *Geochimica et Cosmochimica Acta*, *260*, 161–176. https://doi.org/10.1016/j.gca.2019.06.028

Curtin, D., Beare, M. H., Qiu, W., & Sharp, J. (2019). Does Particulate Organic Matter Fraction Meet the Criteria for a Model Soil Organic Matter Pool? *Pedosphere*, *29*(2), 195–203. https://doi.org/10.1016/S1002-0160(18)60049-9

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Egidi, E., Delgado-Baquerizo, M., Plett, J. M., Wang, J., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., & Singh, B. K. (2019). A few Ascomycota taxa dominate soil fungal communities worldwide. *Nature Communications*, *10*(1), 2369. https://doi.org/10.1038/s41467-019-10373-z

Elith, J., Leathwick, J. R., & Hastie, T. (2008). A working guide to boosted regression trees. *Journal of Animal Ecology*, *77*(4), 802–813. https://doi.org/10.1111/j.1365-2656.2008.01390.x

Fetzer, J., Loeppmann, S., Frossard, E., Manzoor, A., Brödlin, D., Kaiser, K., & Hagedorn, F. (2021). Leaching of Phosphomonoesterase Activities in Beech Forest Soils: Consequences for Phosphorus Forms and Mobility. *Frontiers in Forests and Global Change*, *4*. https://doi.org/10.3389/ffgc.2021.684069

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (Third).

Funk, C. C., Peterson, P. J., Landsfeld, M. F., Pedreros, D. H., Verdin, J. P., Rowland, J. D., Romero, B. E., Husak, G. J., Michaelsen, J. C., & Verdin, A. P. (2014). *A quasi-global precipitation time series for drought monitoring*. https://doi.org/https://doi.org/10.3133/ds832

Gama-Rodrigues, A. C., Sales, M. V. S., Silva, P. S. D., Comerford, N. B., Cropper, W. P., & Gama-Rodrigues, E. F. (2014). An exploratory analysis of phosphorus transformations in tropical soils using structural equation modeling. *Biogeochemistry*, *118*(1–3), 453–469. https://doi.org/10.1007/s10533-013-9946-x

Gomes, M. F., Oliveira, R. L. L. de, Costa, L. R. de J., Campos, W. V. da S., Kato, O. R., Castellani, D. C., & Vasconcelos, S. S. (2024). Soil health indicators in oil palm agroforestry systems in the eastern Amazon, Brazil. *Geoderma Regional*, *37*, e00806. https://doi.org/10.1016/j.geodrs.2024.e00806

Gomes, M. F., Vasconcelos, S. S., Viana-Junior, A. B., Costa, A. N. M., Barros, P. C., Ryohei Kato, O., & Castellani, D. C. (2021). Oil palm agroforestry shows higher soil permanganate

oxidizable carbon than monoculture plantations in Eastern Amazonia. *Land Degradation & Development*, *32*(15), 4313–4326. https://doi.org/10.1002/ldr.4038

Hartmann, M., & Six, J. (2022). Soil structure and microbiome functions in agroecosystems. *Nature Reviews Earth & Environment*, *4*(1), 4–18. https://doi.org/10.1038/s43017-022-00366-w

Hijmans, R., & Phillips, S. (2023). *dismo: Species Distribution Modeling* (1.3-14). https://CRAN.R-project.org/package=dismo

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Hu, J., Du, M., Chen, J., Tie, L., Zhou, S., Buckeridge, K. M., Cornelissen, J. H. C., Huang, C., & Kuzyakov, Y. (2023). Microbial necromass under global change and implications for soil organic matter. *Global Change Biology*. https://doi.org/10.1111/GCB.16676

Indorf, C., Dyckmans, J., Khan, K. S., & Joergensen, R. G. (2011). Optimisation of amino sugar quantification by HPLC in soil and plant hydrolysates. *Biology and Fertility of Soils*, *47*(4), 387–396. https://doi.org/10.1007/s00374-011-0545-5

Jia, S., Liu, X., Lin, W., Li, X., Yang, L., Sun, S., Hui, D., Guo, J., Zou, X., & Yang, Y. (2022). Tree roots exert greater influence on soil microbial necromass carbon than above-ground litter in subtropical natural and plantation forests. *Soil Biology and Biochemistry*, *173*, 108811. https://doi.org/10.1016/j.soilbio.2022.108811

Joergensen, R. G. (2018). Amino sugars as specific indices for fungal and bacterial residues in soil. *Biology and Fertility of Soils*, *54*(5), 559–568. https://doi.org/10.1007/s00374-018-1288-3

Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, *7*(1), 13630. https://doi.org/10.1038/ncomms13630

Kassambara, A. (2023). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests* (0.7.2). https://CRAN.R-project.org/package=rstatix

Kassambara, A., & Mundt, F. (2020). *Extract and Visualize the Results of Multivariate Data Analyses* (1.0.7.999). http://www.sthda.com/english/rpkgs/factoextra

Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., & Kleber, M. (2015). Mineral protection of soil carbon counteracted by root exudates. *Nature Climate Change*, *5*(6), 588–595. https://doi.org/10.1038/nclimate2580

Klose, S., Bilen, S., Ali Tabatabai, M., & Dick, W. A. (2015). *Sulfur Cycle Enzymes* (pp. 125–159). https://doi.org/10.2136/sssabookser9.c7

Kuzyakov, Y., & Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: Concept & review. *Soil Biology and Biochemistry*, *83*, 184–199. https://doi.org/10.1016/j.soilbio.2015.01.025

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vázquez, P. G., Malik, A. A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. E., & Gleixner, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, *6*(1), 6707. https://doi.org/10.1038/ncomms7707

Lavallee, J. M., Soong, J. L., & Cotrufo, M. F. (2020). Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. *Global Change Biology*, *26*(1), 261–273. https://doi.org/10.1111/gcb.14859

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, *25*(1). https://doi.org/10.18637/jss.v025.i01

Leite, M. F. A., Liu, B., Gómez Cardozo, E., Silva, H. R. e, Luz, R. L., Muchavisoy, K. H. M., Moraes, F. H. R., Rousseau, G. X., Kowalchuk, G., Gehring, C., & Kuramae, E. E. (2023). Microbiome resilience of Amazonian forests: Agroforest divergence to bacteria and secondary forest succession convergence to fungi. *Global Change Biology*, *29*(5), 1314–1327. https://doi.org/10.1111/gcb.16556

Lenth, R. (2024). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (1.10.1). https://CRAN.R-project.org/package=emmeans. https://CRAN.R-project.org/package=emmeans

Li, T., Zhang, J., Wang, X., Hartley, I. P., Zhang, J., & Zhang, Y. (2022). Fungal necromass contributes more to soil organic carbon and more sensitive to land use intensity than bacterial necromass. *Applied Soil Ecology*, *176*, 104492. https://doi.org/10.1016/j.apsoil.2022.104492

Li, Y., Shahbaz, M., Zhu, Z., Deng, Y., Tong, Y., Chen, L., Wu, J., & Ge, T. (2021). Oxygen availability determines key regulators in soil organic carbon mineralisation in paddy soils. *Soil Biology and Biochemistry*, *153*, 108106. https://doi.org/10.1016/j.soilbio.2020.108106

Li, Y.-Z., Bao, X.-L., Tang, S.-X., Xiao, K.-Q., Ge, C.-J., Xie, H.-T., He, H.-B., Mueller, C. W., & Liang, C. (2024). Toward soil carbon storage: The influence of parent material and vegetation on profile-scale microbial community structure and necromass accumulation. *Soil Biology and Biochemistry*, *193*, 109399. https://doi.org/10.1016/j.soilbio.2024.109399

Liang, C., Amelung, W., Lehmann, J., & Kästner, M. (2019). Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology*, *25*(11), 3578–3590. https://doi.org/10.1111/gcb.14781

Liang, C., Schimel, J. P., & Jastrow, J. D. (2017). The importance of anabolism in microbial control over soil carbon storage. *Nature Microbiology*, *2*(8), 17105. https://doi.org/10.1038/nmicrobiol.2017.105

Liu, C., Li, X., Mansoldo, F. R. P., An, J., Kou, Y., Zhang, X., Wang, J., Zeng, J., Vermelho, A. B., & Yao, M. (2022). Microbial habitat specificity largely affects microbial co-occurrence patterns and functional profiles in wetland soils. *Geoderma*, *418*, 115866. https://doi.org/10.1016/j.geoderma.2022.115866

Locatelli, J. L., Santos, R. S., Cherubin, M. R., & Cerri, C. E. P. (2022). Changes in soil organic matter fractions induced by cropland and pasture expansion in Brazil's new agricultural frontier. *Geoderma Regional*, *28*, e00474. https://doi.org/10.1016/j.geodrs.2021.e00474

Lopes, A. A. C., Sousa, D. M. G., dos Reis, F. B., Figueiredo, C. C., Malaquias, J. V., Souza, L. M., & Carvalho Mendes, I. (2018). Temporal variation and critical limits of microbial indicators in oxisols in the Cerrado, Brazil. *Geoderma Regional*, *12*, 72–82. https://doi.org/10.1016/j.geodrs.2018.01.003

Lopes, A. A. C. de, Gomes de Sousa, D. M., Chaer, G. M., Bueno dos Reis Junior, F., Goedert, W. J., & de Carvalho Mendes, I. (2013). Interpretation of Microbial Soil Indicators as a Function of Crop Yield and Organic Carbon. *Soil Science Society of America Journal*, *77*(2), 461–472. https://doi.org/10.2136/sssaj2012.0191

Maillard, F., Beatty, B., Park, M., Adamczyk, S., Adamczyk, B., See, C. R., Cavender-Bares, J., Hobbie, S. E., & Kennedy, P. G. (2023). Microbial community attributes supersede plant and soil parameters in predicting fungal necromass decomposition rates in a 12-tree species common garden experiment. *Soil Biology and Biochemistry*, *184*, 109124. https://doi.org/10.1016/j.soilbio.2023.109124

Malik, A. A., Martiny, J. B. H., Brodie, E. L., Martiny, A. C., Treseder, K. K., & Allison, S. D. (2020). Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *The ISME Journal*, *14*(1), 1–9. https://doi.org/10.1038/s41396-019-0510-0

Malik, A. A., Puissant, J., Buckeridge, K. M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H. S., Peyton, J. M., Mason, K. E., van Agtmaal, M., Blaud, A., Clark, I. M., Whitaker, J., Pywell, R. F., Ostle, N., Gleixner, G., & Griffiths, R. I. (2018). Land use driven change in soil pH affects microbial carbon cycling processes. *Nature Communications*, *9*(1), 3591. https://doi.org/10.1038/s41467-018-05980-1

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. https://doi.org/10.14806/ej.17.1.200

Melo, A. S. (2008). O que ganhamos “confundindo” riqueza de espécies e equabilidade em um índice de diversidade? *Biota Neotropica*, *8*(3), 21–27. https://doi.org/10.1590/S1676-06032008000300001

Mendes, L. W., Brossi, M. J. de L., Kuramae, E. E., & Tsai, S. M. (2015a). Land-use system shapes soil bacterial communities in Southeastern Amazon region. *Applied Soil Ecology*, *95*, 151–160. https://doi.org/10.1016/j.apsoil.2015.06.005

Mendes, L. W., Tsai, S. M., Navarrete, A. A., Hollander, M. de, van Veen, J. A., & Kuramae, E. E. (2015b). Soil-Borne Microbiome: Linking Diversity to Function. *Microbial Ecology*, *70*(1), 255–265. https://doi.org/10.1007/s00248-014-0559-2

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, *37*(5), 634–663. https://doi.org/10.1111/1574-6976.12028

Messina, J. P., Brady, O. J., Golding, N., Kraemer, M. U. G., Wint, G. R. W., Ray, S. E., Pigott, D. M., Shearer, F. M., Johnson, K., Earl, L., Marczak, L. B., Shirude, S., Davis Weaver, N., Gilbert, M., Velayudhan, R., Jones, P., Jaenisch, T., Scott, T. W., Reiner, R. C., & Hay, S. I. (2019). The current and future global distribution and population at risk of dengue. *Nature Microbiology*, *4*(9), 1508–1515. https://doi.org/10.1038/s41564-019-0476-8

Moran, E. F., Brondizio, E. S., Tucker, J. M., da Silva-Forsberg, M. C., McCracken, S., & Falesi, I. (2000). Effects of soil fertility and land-use on forest succession in Amazônia. *Forest Ecology and Management*, *139*(1–3), 93–108. https://doi.org/10.1016/S0378-1127(99)00337-0

Nair, P. K. R. (2017). Managed Multi-strata Tree + Crop Systems: An Agroecological Marvel. *Frontiers in Environmental Science*, *5*. https://doi.org/10.3389/fenvs.2017.00088

Navarrete, A. A., Kuramae, E. E., de Hollander, M., Pijl, A. S., van Veen, J. A., & Tsai, S. M. (2013). Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiology Ecology*, *83*(3), 607–621. https://doi.org/10.1111/1574-6941.12018

Navarrete, A. A., Tsai, S. M., Mendes, L. W., Faust, K., de Hollander, M., Cassman, N. A., Raes, J., van Veen, J. A., & Kuramae, E. E. (2015). Soil microbiome responses to the short-term effects of Amazonian deforestation. *Molecular Ecology*, *24*(10), 2433–2448. https://doi.org/10.1111/mec.13172

Nilsson, R. H., Larsson, K.-H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*(D1), D259–D264. https://doi.org/10.1093/nar/gky1022

Oliveira, H. M. R. de, dos Santos, A. C., Leite, R. da C., de Oliveira, L. B. T., da Silva, I. R., & de Oliveira, T. S. (2022b). Repercussion of pastoral systems in C and N fractions stock in northeast Amazonia. *CATENA*, *208*, 105742. https://doi.org/10.1016/j.catena.2021.105742

Oliveira, R. L. L. de, Vasconcelos, S. S., Teixeira, W. G., Viana-Junior, A. B., Castellani, D. C., & Kato, O. R. (2022a). Management Practices Affect Soil Carbon and Physical Quality in Oil Palm Agroforestry Systems in the Amazon. *Journal of Soil Science and Plant Nutrition*, *22*(4), 4653–4668. https://doi.org/10.1007/s42729-022-00947-0

Olivoto, T., & Lúcio, A. D. (2020). metan: An R package for multi-environment trial analysis. *Methods in Ecology and Evolution*, *11*(6), 783–789. https://doi.org/10.1111/2041-210X.13384

Pagès, H., Aboyoun, P., Gentleman, R., & DebRoy, S. (2022). *Biostrings: Efficient manipulation of biological string* (2.66.0). https://bioconductor.org/packages/Biostrings

Pedersen, T. L. (2024). *patchwork: The Composer of Plots* (1.2.0). https://CRAN.R-project.org/package=patchwork

Pedrinho, A., Mendes, L. W., do Rêgo Barros, F. M., Merloti, L. F., Martins e Martins, M., Cotta, S. R., Andreote, F. D., & Tsai, S. M. (2023). Impacts of deforestation and forest regeneration on soil bacterial communities associated with phosphorus transformation processes in the Brazilian Amazon region. *Ecological Indicators*, *146*, 109779. https://doi.org/10.1016/j.ecolind.2022.109779

Pimentel, M. L., de Oliveira, A. B., Schiebelbein, B. E., Carvalho, M. L., Tenelli, S., Cherubin, M. R., Carvalho, J. L. N., Briedis, C., Panosso, A. R., & de Oliveira Bordonal, R. (2024). Quantity, quality and physical protection of soil carbon associated with sugarcane straw removal in southern Brazil. *Soil and Tillage Research*, *237*, 105976. https://doi.org/10.1016/j.still.2023.105976

Pinheiro, J. C., & Bates, D. M. (2023). *nlme: Linear and Nonlinear Mixed Effects Models* (3.1-164). https://CRAN.R-project.org/package=nlme

Pinto, C. R. O., & Nahas, E. (2002). Atividade e população microbiana envolvida nas transformações do enxofre em solos com diferentes vegetações. *Pesquisa Agropecuária Brasileira*, *37*(12), 1751–1756. https://doi.org/10.1590/S0100-204X2002001200011

Prommer, J., Walker, T. W. N., Wanek, W., Braun, J., Zezula, D., Hu, Y., Hofhansl, F., & Richter, A. (2020). Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. *Global Change Biology*, *26*(2), 669–681. https://doi.org/10.1111/gcb.14777

Qian, Z., Li, Y., Du, H., Wang, K., & Li, D. (2023). Increasing plant species diversity enhances microbial necromass carbon content but does not alter its contribution to soil organic carbon pool in a subtropical forest. *Soil Biology and Biochemistry*, *187*, 109183. https://doi.org/10.1016/j.soilbio.2023.109183

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

R Core Team. (2023). *R: A language and environment for statistical computing* (4.2.3). R Foundation for Statistical Computing. https://www.R-project.org/

Rempfert, K. R., Bell, S. L., Kasanke, C. P., Zhao, Q., Zhao, X., Lipton, A. S., & Hofmockel, K. S. (2024). Biomolecular budget of persistent, microbial-derived soil organic carbon: The importance of underexplored pools. *Science of The Total Environment*, *932*, 172916. https://doi.org/10.1016/j.scitotenv.2024.172916

RStudio Team. (2023). *RStudio: Integrated Development Environment for R* (2023.12.0.369). RStudio, PBC. http://www.rstudio.com/

Schauberger, P., & Walker, A. (2023). *openxlsx: Read, Write and Edit xlsx Files* (4.2.5.2). https://CRAN.R-project.org/package=openxlsx

Silva, D. E. O., Costa, R. M., Campos, J. R., Rocha, S. M. B., de Araujo Pereira, A. P., Melo, V. M. M., Oliveira, F. A. S., de Alcantara Neto, F., Mendes, L. W., & Araujo, A. S. F. (2024). Short-term restoration practices change the bacterial community in degraded soil from the Brazilian semiarid. *Scientific Reports*, *14*(1), 6845. https://doi.org/10.1038/s41598-024-57690-y

Slaets, J. I. F., Boeddinghaus, R. S., & Piepho, H. (2021). Linear mixed models and geostatistics for designed experiments in soil science: Two entirely different methods or two sides of the same coin? *European Journal of Soil Science*, *72*(1), 47–68. https://doi.org/10.1111/ejss.12976

Sokol, N. W., Sanderman, J., & Bradford, M. A. (2019). Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. *Global Change Biology*, *25*(1), 12–24. https://doi.org/10.1111/gcb.14482

Sombroek, W. G. (1984). Soils of the Amazon region. In H. Sioli (Ed.), *he amazon, Limnology and Landscape Ecology of a Mighty Tropical River and Its Basin* (Vol. 56, pp. 521–535). https://doi.org/10.1007/978-94-009-6542-3_20

Strickland, M. S., & Rousk, J. (2010). Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, *42*(9), 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Tabatabai, M. A. (1994). Methods of Soil Analysis. In R. W. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai, & A. Wollum (Eds.), *Methods of Soil Analysis, Part 2: Microbiological and Biochemical Properties* (Soil Scien, Vol. 5). Soil Science Society of America. https://doi.org/10.2136/sssabookser5.2

Terasaki Hart, D. E., Yeo, S., Almaraz, M., Beillouin, D., Cardinael, R., Garcia, E., Kay, S., Lovell, S. T., Rosenstock, T. S., Sprenkle-Hyppolite, S., Stolle, F., Suber, M., Thapa, B., Wood, S., & Cook-Patton, S. C. (2023). Priority science can accelerate agroforestry as a natural climate solution. *Nature Climate Change*. https://doi.org/10.1038/s41558-023-01810-5

Tonon-Debiasi, B. C., Debiasi, H., Rondina, A. B. L., Moraes, M. T. de, Franchini, J. C., Balbinot Junior, A. A., Hungria, M., & Nogueira, M. A. (2024). Microbial attributes as structural quality index for physical health of an Oxisol under compaction levels. *Soil and Tillage Research*, *235*, 105872. https://doi.org/10.1016/j.still.2023.105872

Visscher, A. M., Meli, P., Fonte, S. J., Bonari, G., Zerbe, S., & Wellstein, C. (2024). Agroforestry enhances biological activity, diversity and soil-based ecosystem functions in mountain agroecosystems of Latin America: A meta-analysis. *Global Change Biology*, *30*(1). https://doi.org/10.1111/gcb.17036

Vitti, G. C., Otto, R., & Savieto, J. (2015). Manejo do enxofre na agricultura. *Informações Agronômicas*, 1–14. http://www.ipni.net/publication/ia-brasil.nsf/0/871A1FBA4FE913DC83257F31004B6371/$FILE/Page1-14-152.pdf

Wang, B., An, S., Liang, C., Liu, Y., & Kuzyakov, Y. (2021). Microbial necromass as the source of soil organic carbon in global ecosystems. *Soil Biology and Biochemistry*, *162*, 108422. https://doi.org/10.1016/J.SOILBIO.2021.108422

Wang, C., & Kuzyakov, Y. (2024). Soil organic matter priming: The pH effects. *Global Change Biology*, *30*(6). https://doi.org/10.1111/gcb.17349

Wei, J., Zhang, F., Ma, D., Zhang, J., Zheng, Y., Dong, H., Liang, X., Yin, G., Han, P., Liu, M., & Hou, L. (2023). Microbial necromass carbon in estuarine tidal wetlands of China: Influencing factors and environmental implication. *Science of The Total Environment*, *876*, 162566. https://doi.org/10.1016/j.scitotenv.2023.162566

Whalen, E. D., Grandy, A. S., Sokol, N. W., Keiluweit, M., Ernakovich, J., Smith, R. G., & Frey, S. D. (2022). Clarifying the evidence for microbial- and plant-derived soil organic matter, and the path toward a more quantitative understanding. *Global Change Biology*, *28*(24), 7167–7185. https://doi.org/10.1111/gcb.16413

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Wickham, H., & Bryan, J. (2023). *readxl: Read Excel Files* (1.4.3). https://CRAN.R-project.org/package=readxl

Wirth, S., Hohn, A., & Muller, L. (2008). Translocation of Soil Enzyme Activity by Leachates from Different Agricultural Drainage Systems. *International Journal of Soil Science*, *3*(2), 52–61. https://doi.org/10.3923/ijss.2008.52.61

Xia, Q., Rufty, T., & Shi, W. (2020). Soil microbial diversity and composition: Links to soil texture and associated properties. *Soil Biology and Biochemistry*, *149*, 107953. https://doi.org/10.1016/j.soilbio.2020.107953

Xu, F., Chen, J., Yang, Y., Wu, J., Li, C., Chen, Y., Wan, X., Luo, G., Zhang, Y., Li, S., & Su, J. (2023). Sowing crop affects soil microbial necromass carbon via altering soil fungal community structure in a macadamia-based agroforestry system. *Ecological Indicators*, *157*, 111262. https://doi.org/10.1016/j.ecolind.2023.111262

Yang, Y., Dou, Y., Wang, B., Wang, Y., Liang, C., An, S., Soromotin, A., & Kuzyakov, Y. (2022). Increasing contribution of microbial residues to soil organic carbon in grassland restoration chronosequence. *Soil Biology and Biochemistry*, *170*, 108688. https://doi.org/10.1016/j.soilbio.2022.108688

Zhao, X., Hao, C., Zhang, R., Jiao, N., Tian, J., Lambers, H., Liang, C., Cong, W.-F., & Zhang, F. (2023). Intercropping increases soil macroaggregate carbon through root traits induced microbial necromass accumulation. *Soil Biology and Biochemistry*, *185*, 109146. https://doi.org/10.1016/j.soilbio.2023.109146

Zhou, R., Liu, Y., Dungait, J. A. J., Kumar, A., Wang, J., Tiemann, L. K., Zhang, F., Kuzyakov, Y., & Tian, J. (2023). Microbial necromass in cropland soils: A global meta-analysis of management effects. *Global Change Biology*, *29*(7), 1998–2014. https://doi.org/10.1111/gcb.16613

Supplementary Material

Table S1. History of fertilizer and limestone application in oil palm-based agroforestry systems in of Tomé-Açu (state of Pará, PA), located in the Amazon biome and the Northern region of Brazil (Lat.: 02° 40' 54" S; Long.: 48° 16' 11" W). **Source** Alessa Mendanha (Costa et al., 2023).

Lime/fertilizer	Unit	Oil palm (AFS)											Strip under integration and diversification (SID) (AFS)		
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2008	2017	2018
Limestone [d]	kg ha^{-1}										1716		1300 [b]	1716	
ARAD reactive rock phosphate [e]	kg plant^{-1}	0.3		1	1.5						0.7	2.5	0.3 [a]	0.6 [a]/0.7 [c]	0.6 [a]/0.9 [c]
Chaff charcoal [f]	kg plant^{-1}	10											10 [b]		
Chicken manure [g]	kg plant^{-1}												10 [b]		
Bone meal [h]	kg plant^{-1}		1	0.5	0.5										
Castor cake [i]	kg plant^{-1}		2	2	4										
Oil palm cake [j]	kg plant^{-1}									30	30	40		1 [a]/2.5 [c]	
FTE BR12 [k]	kg plant^{-1}			0.3	0.2										
Empty fruit bunches of oil palm [l]	kg plant^{-1}			100	221	150	200	240	240						
Borax [m]	kg plant^{-1}	10		0.1											
Organic compost [n]	kg plant^{-1}		40										40 [b]		
Yoorin [o]	kg plant^{-1}														
Produbor 10 [p]	kg plant^{-1}										0.1	0.15	0.1 [c]	0.1 [a]	
Potassium polysulfate [q]	kg plant^{-1}										2.6	4.5		0.17 [a]/0.7 [c]	
Potassium sulfate [r]	kg plant^{-1}	40					0.8	0.8	0.4	1.8		1		0.1 [a]/0.3 [c]	0.15[a]/0.5[c]

[a] *Theobroma cacao*
[b] Fertilizer applied over the entire strip
[c] *Euterpe oleracea* Mart.
[d] Mg: 12%; Ca: 38%
[e] P: 10%; Ca: 37%
[f] K: 5.9%; Mg: 2.8%; Ca: 15.40%
[g] N: 2.58%; P: 2.27%; K: 2.31%; Mg: 0.44%; Ca: 30%
[h] N: 2%; P: 20%; K: 0.12%; Mg: 0.24%; Ca: 30%
[I] N: 5.44%; P: 1.91%; K: 1.54%; Mg: 0.5%; Ca: 1.8%
[J] N: 2.4%; P: 0.56%; K: 0.61%; Mg: 0.01%; Ca: 4%; S: 0.4%; Zn: 0.01%; B: 0.05%
[l] B: 1.8%; Cu: 0.85%; Mn: 2.0%; Zn: 9.0%; S: 3.9%
[l] N: 0.33%; P: 0.04%; K: 0.55%; Mg: 0.09%; Ca:0.28%; S: 0.04%
[m] B: 20.8%; B2O3: 67%
[n] N: 0.80%; P: 0.40%; K: 0.30%; Mg: 0.10%; Ca: 0.23%; S: 0.03%
[o] P: 16%; Mg: 7%; Ca: 18%; S: 6%; Zn: 0.55%; B: 0.1%
[p] B: 10%
[q] K: 14.0%; Mg: 3.5%; Ca: 12%; S: 19%
[r] K: 50%; S: 18%

Table S2. Table S2. Soil physicochemical parameters according to the 8 sampling points (2 in CA and 6 in AFS), sampling station and soil depth. Data shown are mean ± standar error (SE) (n = 4).

Land Use	Sampling Point	Season	pH	Ca^{2+} (mmolc.dm·³)	Mg^{2+} (mmolc.dm·³)	K^{+} (mmolc.dm·³)	H + Al^{3+} (mmolc.dm·³)	Clay (%)	Silt (%)	Sand (%)
Depth: 0-10 cm										
CA	CA-1	Dry	4.24 ± 0.25	11.0 7 ±5.28	1.12 ± 0.67	0.22 ± 0.09	37.45 ± 4.47	23.79 ± 3.30	4.95± 3 .15	71.26 ± 5.09
		Rainy	4.11 ± 0.11	9.67 ± 0.90	1.80 ± 0.23	0.50 ± 0.10	40.45 ± 3.84			
	CA-2	Dry	4.64 ± 0.29	22.5 8 ±9.06	1.48 ± 1.11	0.32 ± 0.11	34.67 ± 5.51	22.35 ± 5.23	7.07± 1 .80	70.58 ± 6.36
		Rainy	4.17 ± 0.25	12.1 0 ±6.23	1.27 ± 0.53	0.30 ± 0.08	37.77 ± 2.52			
AFS	AFS-1	Dry	5.10 ± 0.24	47.6 0 ±14.14	7.90 ± 0.94	1.16 ± 0.93	36.20 ± 5.33	26.97 ± 5.68	12.39 ± 3.02	60.64 ± 6.91
		Rainy	4.80 ± 0.07	30.8 8 ±1.56	4.47 ± 0.41	0.74 ± 0.04	30.15 ± 3.34			
	AFS-2	Dry	4.61 ± 0.30	17.4 2 ±7.42	1.80 ± 0.80	0.31 ± 0.06	37.83 ± 6.03	25.39 ± 4.44	10.38 ± 2.51	64.23 ± 6.80
		Rainy	4.66 ± 0.24	26.6 2 ±6.40	4.38 ± 2.43	0.39 ± 0.10	34.77 ± 1.30			
	AFS-3	Dry	4.96 ± 0.20	32.7 7 ±7.22	9.42 ± 2.28	0.50 ± 0.03	30.00 ± 4.89	26.92 ± 3.69	9.71± 3 .73	63.37 ± 5.03
		Rainy	4.84 ± 0.11	35.4 8 ±1.64	7.35 ± 1.25	0.64 ± 0.06	32.23 ± 4.15			
	AFS-4	Dry	5.06 ± 0.19	43.6 2 ±11.30	9.60 ± 1.04	0.58 ± 0.13	31.50 ± 7.42	22.89 ± 2.06	15.55 ± 4.78	61.56 ± 3.65
		Rainy	4.95 ± 0.21	42.7 0 ±2.41	7.80 ± 0.21	0.86 ± 0.28	30.30 ± 6.51			
	AFS-5	Dry	5.17 ± 0.27	42.9 0 ±6.91	10.4 3 ±0.98	0.94 ± 0.75	28.37 ± 7.34	24.92 ± 3.90	14.05 ± 6.03	61.03 ± 3.58
		Rainy	5.24 ± 0.07	56.9 8 ±9.69	11.1 2 ±0.68	1.31 ± 0.25	26.05 ± 2.43			
	AFS-6	Dry	4.97 ± 0.12	24.6 8 ±9.03	8.42 ± 0.70	0.71 ± 0.29	28.73 ± 4.75	23.83 ± 3.83	15.25 ± 4.29	60.92 ± 5.93
		Rainy	5.10 ± 0.05	38.4 8 ±5.36	10.4 3 ±1.32	0.80 ± 0.15	28.12 ± 2.06			
Depth: 10-20 cm										
CA	CA-1	Dry	4.04 ± 0.18	5.95 ± 3.57	0.30 ± 0.20	0.14 ± 0.09	32.7 2 ±2.80	31.30 ± 4.96	6.45 ± 2.57	62.25 ± 2.72
		Rainy	3.88 ± 0.02	3.48 ± 0.56	0.60 ± 0.17	0.65 ± 0.14	42.4 8 ±3.77			
	CA-2	Dry	4.22 ± 0.22	9.97 ± 4.40	0.58 ± 0.16	0.26 ± 0.04	36.6 2 ±9.26	32.33 ± 5.87	4.88± 2 .40	62.79 ± 6.46
		Rainy	4.00 ± 0.12	6.70 ± 3.88	0.68 ± 0.33	0.24 ± 0.09	40.7 0 ±3.29			
AFS	AFS-1	Dry	5.00 ± 0.22	25.9 7 ±7.94	4.97 ± 0.36	0.83 ± 0.11	29.8 0 ±5.70	32.35 ± 6.32	11.40 ± 2.95	56.25 ± 7.86
		Rainy	4.34 ± 0.14	13.2 0 ±3.02	2.48 ± 0.41	0.56 ± 0.14	30.5 2 ±0.93			
	AFS-2	Dry	4.96 ± 0.11	18.2 3 ±2.76	2.30 ± 1.06	0.24 ± 0.10	23.2 8 ±4.19	35.28 ± 1.13	11.87 ± 1.81	52.85 ± 2.49
		Rainy	4.67 ± 0.08	19.2 3 ±3.66	4.12 ± 1.71	0.65 ± 0.20	29.0 5 ±6.94			
	AFS-3	Dry	4.63 ± 0.25	13.6 8 ±1.82	5.62 ± 1.17	0.40 ± 0.01	29.8 2 ±7.84	33.91 ± 2.16	6.42± 2.60	59.68 ± 3.80
		Rainy	4.50 ± 0.08	16.3 2 ±3.68	3.75 ± 0.50	0.58 ± 0.18	31.1 5 ±2.17			
	AFS-4	Dry	4.80 ± 0.21	19.0 5 ±5.57	5.78 ± 0.53	0.35 ± 0.09	25.9 5 ±4.10	31.80 ± 5.30	6.08± 3.25	62.13 ± 5.91
		Rainy	4.74 ± 0.25	18.5 0 ±6.26	4.70 ± 1.08	0.74 ± 0.22	24.3 0 ±1.22			
	AFS-5	Dry	4.88 ± 0.15	23.4 8 ±4.81	5.58 ± 0.78	0.61 ± 0.13	29.7 5 ±3.64	30.40 ± 4.09	6.11± 3.22	63.49 ± 4.67
		Rainy	5.00 ± 0.14	30.3 2 ±7.68	6.80 ± 0.90	0.83 ± 0.30	24.9 0 ±1.81			
	AFS-6	Dry	4.31 ± 0.20	11.0 5 ±4.60	3.78 ± 1.15	0.52 ± 0.20	28.9 5 ±2.38	36.25 ± 3.96	9.58± 2.36	54.17 ± 6.27
		Rainy	4.52 ± 0.14	14.7 8 ±2.11	6.18 ± 0.93	0.74 ± 0.41	28.6 2 ±4.94			
Depth: 20-30 cm										
CA	CA-1	Dry	4.09 ± 0.22	4.75 ± 3.99	0.80 ± 0.50	0.06 ± 0.02	26.8 0 ±2.11	38.89 ± 5.20	6.32± 3 .11	54.79 ± 4.70
		Rainy	3.90 ± 0.03	2.72 ± 0.41	0.43 ± 0.39	0.77 ± 0.41	35.3 8 ±5.39			
	CA-2	Dry	4.06 ± 0.05	6.45 ± 3.19	0.55 ± 0.61	0.20 ± 0.06	34.9 8 ±4.12	39.85 ± 7.02	1.98± 0.80	58.17 ± 7.49
		Rainy	3.94 ± 0.01	3.80 ± 1.22	0.40 ± 0.20	0.19 ± 0.07	33.5 5 ±1.82			
AFS	AFS-1	Dry	4.88 ± 0.39	19.1 0 ±8.26	3.60 ± 0.80	0.21 ± 0.10	27.0 0 ±5.00	40.35 ± 3.72	9.59± 3.05	50.05 ± 4.85
		Rainy	4.16 ± 0.18	8.23 ± 4.09	1.98 ± 0.90	0.60 ± 0.38	32.5 5 ±4.21			
	AFS-2	Dry	4.95 ± 0.18	18.1 8 ±6.86	2.78 ± 0.75	0.27 ± 0.07	20.3 2 ±3.28	41.68 ± 2.27	11.13 ± 2.25	47.20 ± 2.54
		Rainy	4.32 ± 0.16	10.4 3 ±1.72	2.38 ± 0.78	0.38 ± 0.06	28.5 3 ±6.19			
	AFS-3	Dry	4.24 ± 0.17	6.90 ± 0.92	2.65 ± 0.71	0.29 ± 0.04	31.2 3 ±3.63	42.82 ± 3.38	8.18± 4.91	49.00 ± 5.53

		Rainy	4.15 ± 0.04	8.43 ± 0.70	2.10 ± 0.41	0.27 ± 0.11	33.7 0 ±1.78		
	AFS-4	Dry	4.70 ± 0.27	15.3 2 ±2.79	3.95 ± 0.36	0.38 ± 0.16	22.3 3 ±5.25	41.86 ± 3.65	7.24± 3.96	50.90 ± 5.37
		Rainy	4.34 ± 0.23	10.2 5 ±4.68	3.58 ± 1.24	0.52 ± 0.11	27.4 2 ±2.77		
	AFS-5	Dry	4.45 ± 0.27	11.8 5 ±5.94	3.08 ± 1.18	0.22 ± 0.11	29.5 5 ±3.60	42.37 ± 4.96	8.33± 3.94	49.29 ± 2.87
		Rainy	4.38 ± 0.28	11.2 8 ±5.62	3.75 ± 1.07	0.63 ± 0.28	28.4 2 ±2.94		
	AFS-6	Dry	4.46 ± 0.25	12.6 0 ±5.01	4.00 ± 1.97	0.66 ± 0.36	27.2 5 ±4.07	34.82 ± 15.87	18.68 ± 15.71	46.50 ± 2.14
		Rainy	4.11 ± 0.07	5.22 ± 1.35	2.30 ± 0.87	0.22 ± 0.07	32.5 5 ±4.59		

*CA: conventional agriculture; and AFS: agroforestry system. The other abbreviations are explained in the “Methods section”, Table 1.

Table S3. Historical precipitation (last 42 years) in Tomé-Açu (state of Pará, PA), located in the Amazon biome and the Northern region of Brazil (Lat.: 02° 40' 54" S; Long.: 48° 16' 11" W). Source: CHIRPS: Rainfall Estimates from Rain Gauge and Satellite Observations (Funk et al., 2014).

Yer	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual accumulation
	Unit: mm												
1981	318.37	361.83	295.47	149.98	228.42	58.61	63.98	60.54	38.04	47.63	113.17	168.86	1904.89
1982	414.58	511.77	521.33	358.39	156.37	60.04	51.67	66.18	58.76	72.17	79.59	92.7	2443.55
1983	113.44	291.99	303.7	201.13	130.84	66.17	55.41	55.59	53.51	76.09	36.24	250.42	1634.54
1984	393.53	451.91	515.25	491.46	498.19	110.41	123.28	101.91	138.19	140.04	109.81	138.18	3212.18
1985	453.46	501.88	495.82	337.45	378.15	117.59	81.15	95.59	70.5	158.47	197.13	217.08	3104.25
1986	304.8	393.5	457.35	518.64	203.63	147.7	85.72	43.59	62.08	80.12	191.05	187.31	2675.50
1987	249.77	259.79	485	308.17	154.32	143.17	53.09	66.49	87.34	43.03	46.65	125.2	2022.01
1988	410.65	318.65	512.94	426.1	273.95	169.77	67.04	92.36	89.36	87.41	127.81	295.95	2872.00
1989	271.53	456.12	488.39	438.14	365.95	155.64	110	82.11	72.71	162.32	89.74	269.69	2962.34
1990	175.83	380.62	379.57	315.14	158.42	104.93	148.59	38.76	51.18	35.55	128.9	150.17	2067.67
1991	387.51	264.53	358.26	310.67	228.72	119.31	79.92	32.29	25.49	84.35	14.94	75.78	1981.76
1992	214.05	273.64	347	216.58	89.78	53.03	48.7	55.35	46.05	17	72.81	91.5	1525.48
1993	254.67	312.31	474.68	313.36	188.14	77.56	67.88	73.37	71.55	117.1	186.23	179.54	2316.40
1994	376.94	394.19	511.99	453.87	371.39	191.31	74.83	66.96	79.26	70.07	64.49	145.17	2800.48
1995	223.15	363.33	275.2	392.73	453.09	58.02	76.19	26.71	32.63	48.05	156.68	175.9	2281.69
1996	367.19	394.07	549.32	385.58	338.83	61.28	89.2	98.62	52.22	93.9	110.61	144.08	2684.89
1997	366.44	240.4	432.48	359.68	154.22	23.66	34.48	73.55	12.86	59.51	127.12	118.38	2002.78
1998	403.07	175.98	452.54	325.51	173.46	106.94	77.03	70.93	31.08	85.16	211.09	256.38	2369.17
1999	324.64	420.01	541.9	440.78	413.66	89.46	61.18	52.29	115.48	99.43	142.75	262.24	2963.82
2000	371.83	456.67	481.52	412.84	316.96	141.55	197.45	76.24	110.39	43.43	42.24	124.11	2775.22
2001	493.9	456.93	509.68	384.76	243.37	229.28	99.15	19.61	72.39	80.7	54.08	140.95	2784.78
2002	409.55	229.89	448.79	474.8	282.36	154.52	83.11	66.76	36.21	70.75	70.87	156.04	2483.65
2003	174.96	361.72	479.21	322.53	204.8	119.16	71.68	88.02	79.24	98.3	80.02	167.54	2247.18
2004	368.98	455.08	471.3	360.67	166.66	92.45	112.95	104.48	131.09	120.9	41.39	145.61	2571.56
2005	224.92	392.9	398.84	398.69	304.97	90.08	50.66	55.87	23.79	71.7	112.07	339.31	2463.81
2006	346.15	251.77	472.63	531.49	409.77	61.98	68.4	60.97	85.38	88.76	243.58	266.3	2887.19

2007	202.4	478.27	496.52	444.88	304.47	89	78.15	41.88	33.4	107.88	94.26	280.53	2651.63
2008	441.26	394.19	564.31	438.27	366.33	143.44	45.75	52.45	85.01	85.29	71.87	216.99	2905.15
2009	349.1	459.12	569.97	473.09	591.39	220.93	74.04	43.21	37.92	49.47	31.61	257.66	3157.51
2010	299.98	283.52	260.11	434.34	287.75	89.82	91.29	78.74	38.52	141.14	133.19	212.81	2351.22
2011	396.64	541.57	541.19	376.59	276.99	84.43	93.64	91.09	42.83	154.68	195.42	165.55	2960.61
2012	351.58	464.43	428.82	322.48	188.24	121.5	80.67	55.64	34.59	56.24	86.1	211.17	2401.47
2013	350.96	497.59	443.44	371.77	340.32	158.45	144.07	102.72	67.39	94.14	312.03	208.77	3091.65
2014	434.5	546.67	523.39	456.57	267.26	219.42	120.71	101.76	101.17	110.84	55.38	214.22	3151.89
2015	403.18	393.3	492.03	404.69	315.2	148.94	133.73	28.05	52.95	49.25	33.78	200.64	2655.74
2016	226.13	339.54	547.42	366.76	378.89	117.24	90.29	67.41	68.71	222.67	56.54	310.65	2792.24
2017	587.91	589.64	561.67	379.05	253.8	113.75	40.98	82.66	69.01	111.96	50.42	270.61	3111.45
2018	272.82	452.69	399.42	376.58	442.29	111.46	87.17	141.89	113.48	59.81	250.67	400.35	3108.63
2019	464.11	463.47	637.17	475.71	344.32	107.37	115.63	76.13	121.95	101.66	196.1	330.78	3434.40
2020	417.58	437.9	556.26	363.13	233.93	177.44	60.28	19.8	99.85	177.25	403.52	154.32	3101.26
2021	286.39	419.67	498.09	347.04	276.38	138.82	194.57	96.04	159.47	152.69	265.67	219.16	3054.01
2022	347.91	266.91	599.22	340.83	361.94	157.46	77.31	66.03	66.25	65.06	254.72	313.41	2917.04
2023	315.24	411.46	542.25	306.7	243.64	133.06	50.35	22.45	15.76	71.77	126.64	169.71	2409.03
Mean (42 years)	338.64	390.96	472.59	379.25	287.48	119.45	86.31	67.28	68.26	92.18	127.19	205.16	2634.74
SE (42 years)	14.64	14.71	13.18	12.14	16.32	7.25	5.56	4.10	5.29	6.52	13.08	11.28	68.58

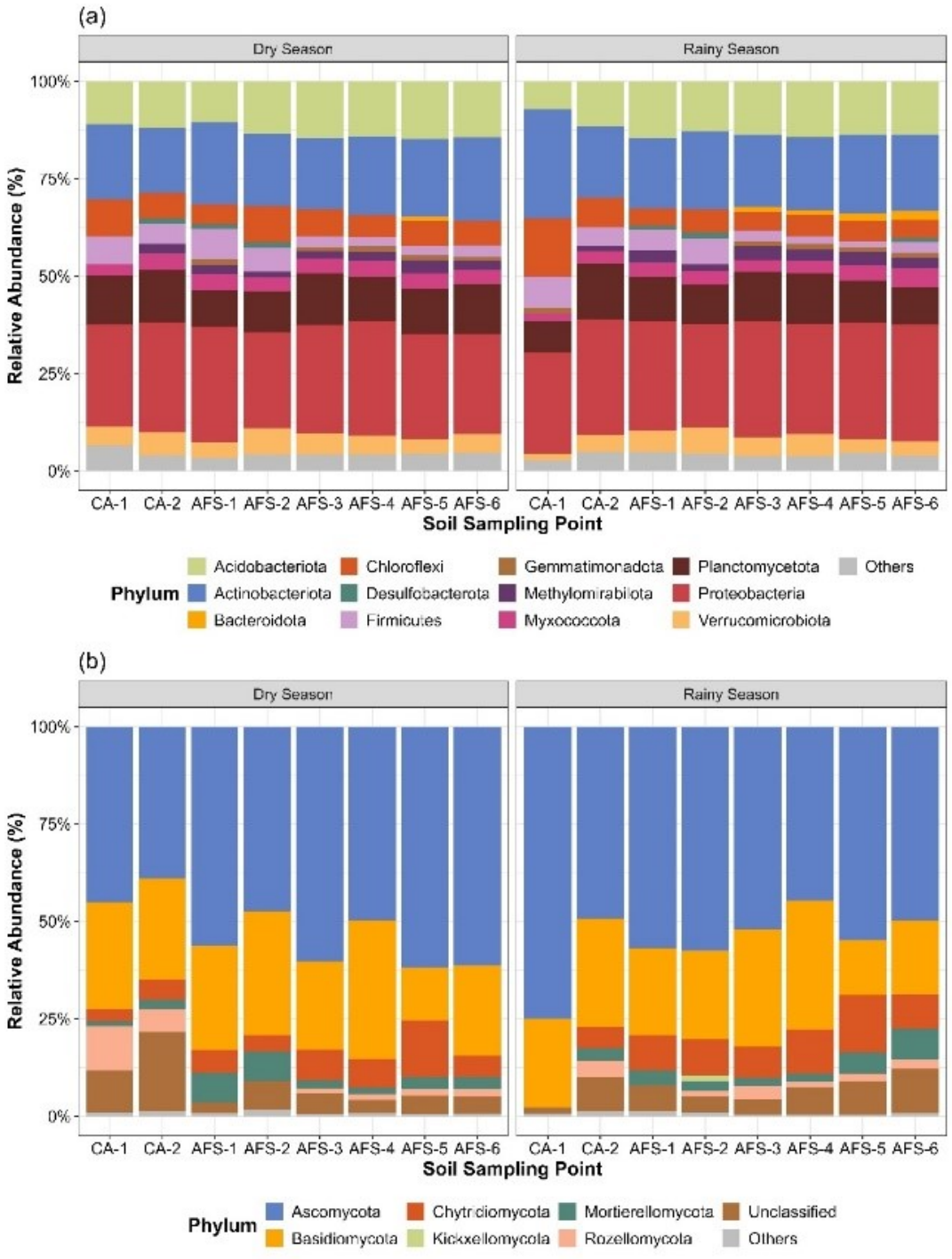

Figure S1. Relative abundance (%) of main bacteria (a) and fungi phyla (b) according to the 8 sampling points (2 in CA and 6 in AFS) and sampling season at 0-10 cm depth. Others: low abundant (< 1%); and Unclassified: there was no outcome in the taxonomic affiliation with the UNITE database at 99% similarity. Abbreviations are explained in the “Methods section”, Table 1.

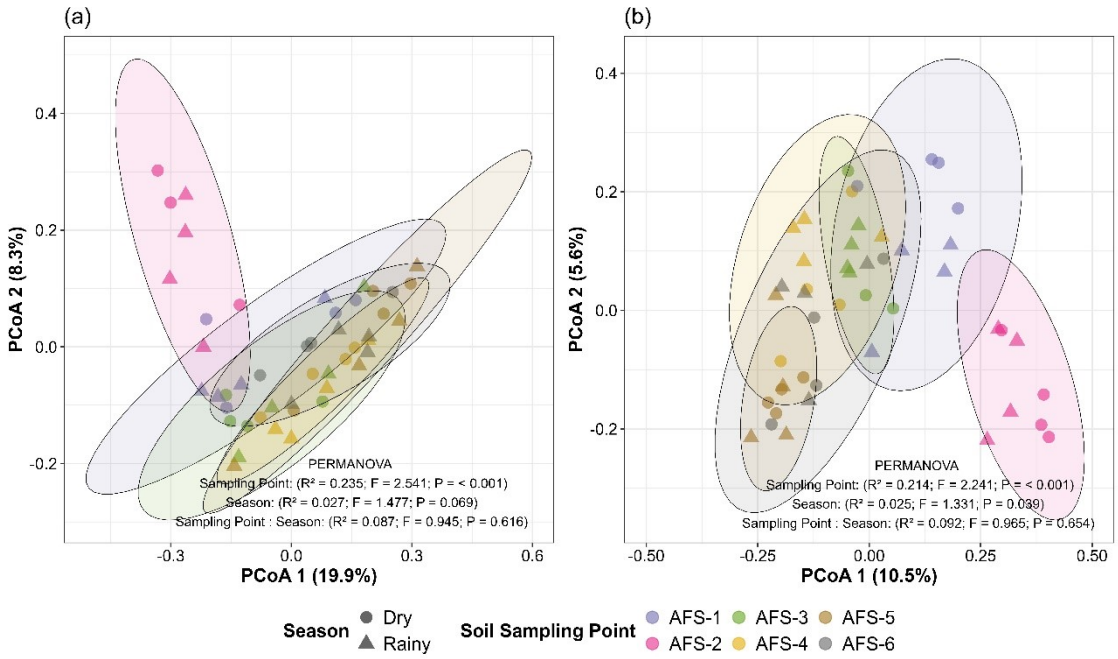

Figure S2. Community structure of bacteria (a) and fungi (b) revealed by principal coordinates analysis (PCoA) with only 6 sampling points in AFS and sampling season at depth 0-10 cm. Ellipses symbolize the dispersion of samples within each sampling point at 95% confidence. Abbreviations are explained in the “Methods section”, Table 1.

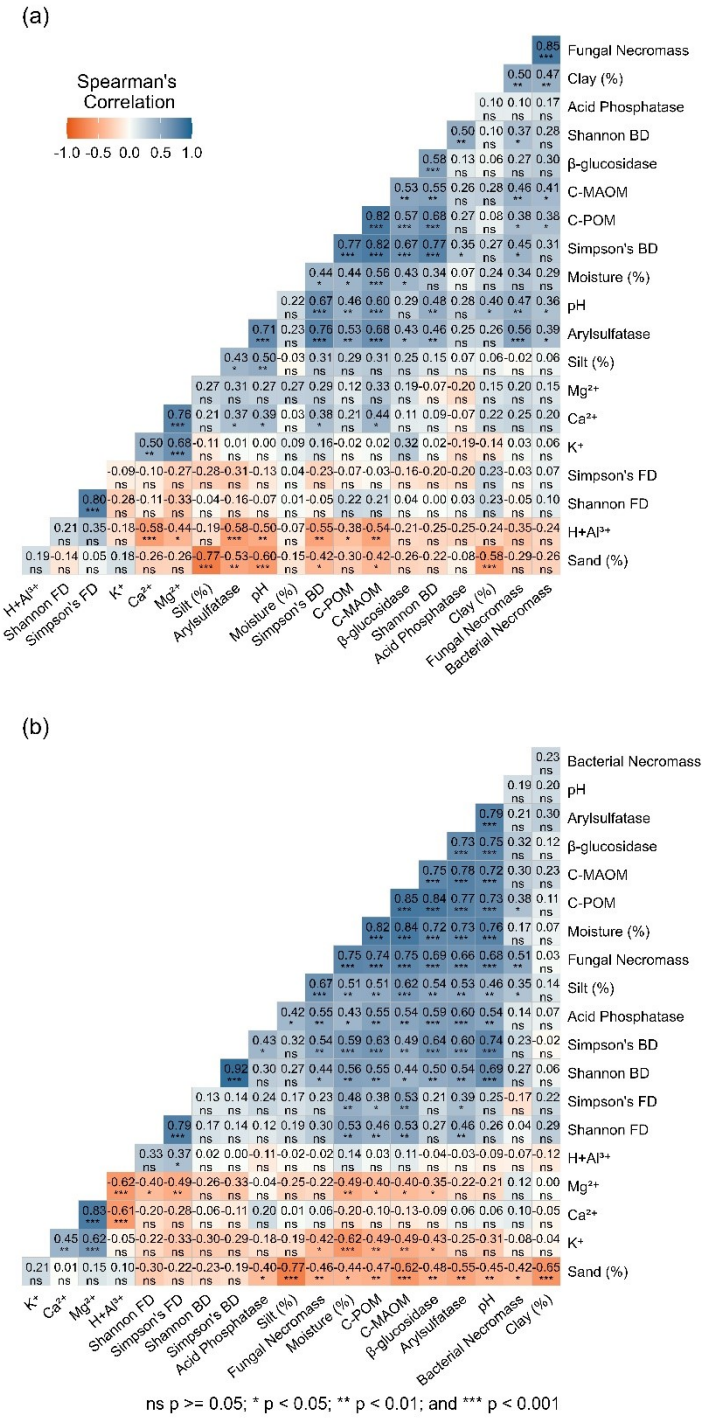

Figure S3. Spearman's correlation coefficient among the biological, chemical, and physical attributes in carbon pools in the dry (a) and rainy season. Asterisks indicates significant correlation between each variable assessed. C-POM: carbon content present in the > 53 µm physical fraction of soil organic matter (SOM); C-MAOM: carbon content present in the < 53 µm physical fraction of SOM; pH: soil hydrogen potential; $H+Al^{3+}$: aluminium saturation in the soil; Simpson's BD: Simpson's bacterial diversity index; Simpson's FD: Simpson's fungal diversity index; Ca^{2+}: calcium content in the soil; Shannon BD: Shannon's bacterial diversity index; Mg^{2+}: magnesium content in the soil; Shannon FD: Shannon's fungal diversity index; K^{+}: potassium content in the soil.

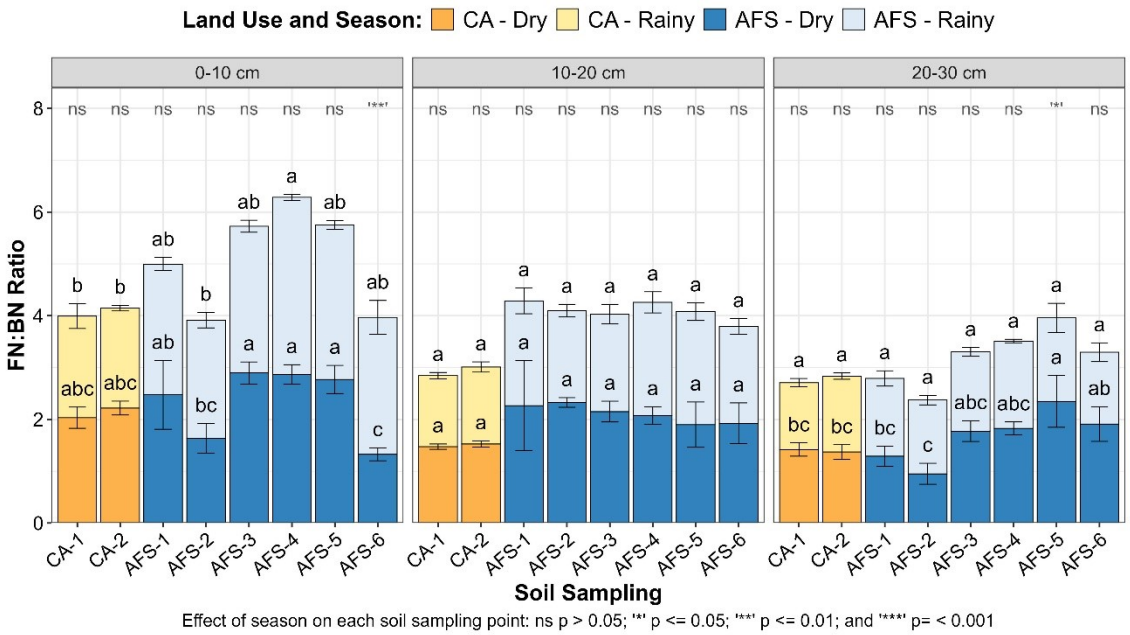

Figure S4. Fungal Necromass:Bacterial Necromass ratio (FN:BN Ratio) according to the 8 sampling points (2 in CA and 6 in AFS), sampling season, and soil depth. Mean values followed by the same letter do not differ significantly among sampling points according to Tukey's test ($p \leq 0.05$). Significance codes for differences within sampling points due to sampling season: ns $p > 0.05$, '*' $p \leq 0.05$, '**' $p \leq 0.01$, and '***' $p \leq 0.001$. Values are reported as mean ± standard error (n = 5). Abbreviations are explained in the "Methods section", Table 1.

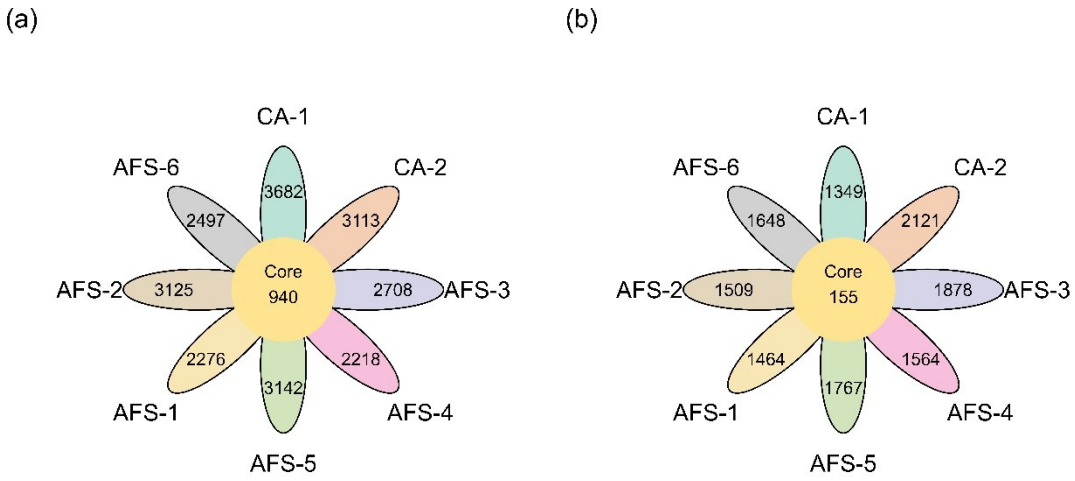

Figure S5. Venn diagram representing the bacterial (a) and fungal (b) Amplicon Sequence Variant (ASV) (99% similarity) shared among the 8 sampling points (2 in CA and 6 in AFS). Abbreviations are explained in the "Methods section", Table 1.