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TROPICAL**

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Mudanças impulsionadas pela idade na composição funcional, junto à diversidade funcional, moldam a prestação de serviços ecossistêmicos em sistemas agroflorestais

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Dissertação apresentada ao Curso de Pós-Graduação em Ciências Biológicas – Botânica Tropical, da Universidade Federal Rural da Amazônia (UFRA), como parte dos requisitos para obtenção do título de Mestre.

Orientadora: Prof.^a Dra. Grazielle Sales Teodoro

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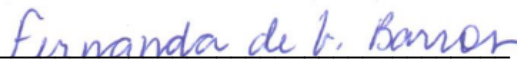
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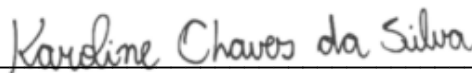
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Dedico este trabalho a quem tem o brio ferido pelo
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“No começo pensei que estava lutando para salvar seringueiras, depois pensei que estava lutando para salvar a floresta amazônica, agora percebo que estou lutando pela humanidade” (Chico Mendes).

RESUMO

A intensificação do uso da terra simplificou a biodiversidade funcional em ecossistemas tropicais, prejudicando a prestação de serviços ecossistêmicos. Embora os sistemas agroflorestais (SAFs) tenham sido amplamente promovidos como alternativas sustentáveis ao desmatamento, ainda não está claro como a idade dos sistemas influencia as estratégias ecológicas das árvores e a diversidade funcional e como esses padrões funcionais afetam a prestação de serviços ecossistêmicos. Avaliamos as características funcionais e a diversidade de espécies arbóreas em oito SAFs na Amazônia Ocidental, com diferentes idades. Medimos características acima e abaixo do solo ligadas a estratégias ecológicas e indicadores de serviços ecossistêmicos. Avaliamos como a idade do sistema afeta a identidade funcional, a complementaridade de características e a multifuncionalidade, e a influência desses padrões funcionais na prestação de serviços ecossistêmicos. Nossas descobertas revelam que a composição funcional das comunidades de plantas impulsionada pelo homem muda sistematicamente com a idade do SAF, promovendo a fertilidade e proteção do solo e o armazenamento de biomassa acima do solo, o que pode reduzir os insumos externos e aumentar a resiliência climática. Além disso, em SAFs de longo prazo, os atributos funcionais convergiram para estratégias conservadoras, que suportam a multifuncionalidade do ecossistema e podem aumentar a estabilidade enquanto reduzem os custos de gerenciamento. A identidade funcional, especificamente ao teor de matéria seca foliar, também foi associada a solos mais protegidos em SAFs mais antigos. Isso reforça a necessidade de considerar atributos funcionais no planejamento e na política agroflorestal, o que pode melhorar a resiliência e a prestação de serviços ecossistêmicos de longo prazo, particularmente em áreas tropicais degradadas, fortalecendo o papel da agrossilvicultura na agricultura sustentável.

Palavras-chave: Agroecologia; atributo; complementaridade; ecossistema tropical; multifuncionalidade.

ABSTRACT

Land-use intensification has simplified functional biodiversity in tropical ecosystems, undermining the provision of ecosystem services. Although agroforestry systems (AFS) have been widely promoted as sustainable alternatives to deforestation, it remains unclear how systems' age influence plant ecological strategies and functional diversity, and how these functional patterns affect the provision of ecosystem services. We evaluated the functional traits and diversity of tree species across eight AFSs in western Amazonia, differing in age. We measured above- and below-ground traits linked to plant ecological strategies, and ecosystem service proxies. We assessed how system age affects functional identity, trait complementarity, and multifunctionality, and how these functional patterns influence the provision of ecosystem services. Our findings reveal that the human-driven functional composition of tree communities shifts systematically with AFS age, promoting soil fertility and protection, and aboveground biomass storage, which may reduce external inputs and enhance climate resilience. Also, in long-term AFSs, functional traits converged toward conservative strategies, which support ecosystem multifunctionality and may enhance stability while reducing management costs. Functional identity, specifically to leaf dry matter content, was also associated with more protected soils in long-term AFSs. This reinforces the need to consider functional traits in agroforestry planning and policy, which can improve resilience and long-term ecosystem service provision, particularly in degraded tropical areas, strengthening the role of agroforestry in sustainable agriculture.

Keyword: agroecology; complementarity; multifunctionality; trait; tropical ecosystem

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1. FUNDAMENTAÇÃO TEÓRICA

1.1 Sistemas Agroflorestais

Os SAFs são sistemas de uso da terra e das tecnologias, onde plantas lenhosas perenes são utilizadas nas mesmas unidades de manejo da terra com culturas agrícolas e /ou animais, utilizando diversas formas de arranjo espacial ou sequência temporal (Nair 1993; Romano et al. 2019). Esses sistemas refletem os interesses de cada proprietário, culminando em variação nos arranjos de espécies, conforme as preferências dos proprietários, relacionando-se com as necessidades de segurança alimentar, demandas do mercado e suas mudanças, condições edafoclimáticas da região, bem como as necessidades ecológicas e de conservação de cada um dos locais (Almeida et al. 2012; Vineeta et al. 2022).

Esse modelo de cultivo é cada vez mais entendido como uma estratégia fundamental de adaptação às alterações climáticas e aliado da segurança alimentar (Tscharntke et al. 2012; Isaac & Borden 2020). Os benefícios desse tipo de sistema são mais amplos que na agricultura tradicional, proporcionando melhorias na paisagem, regulação do microclima, bem como estabilidade da biodiversidade, restauração e preservação do solo, maior retorno de nutrientes ao solo sob serrapilheira (Pezarico et al. 2013; Souza & Piña-Rodrigues 2013; Camargo et al. 2017; Vineeta et al. 2022; Martins et al. 2023).

Em um cenário no qual 33% dos solos do mundo encontram-se degradados, os SAFs constituem um instrumento relevante na conservação, restauração e melhoria ambiental. A presença de espécies arbóreas em campos agrícolas traz muitos benefícios como o aumento na taxa de associações micorrízicas, que aumenta produtividade (Beule et al. 2020); culmina no aumento do estoque de carbono e nutrientes do solo (Pardon et al. 2017) e outros benefícios como sequestro de carbono, enriquecimento do solo, melhorias na qualidade do ar e produtividade (Sollen-Norrlin et al. 2020; Cardinael et al. 2021). No entanto, abordagens baseadas nas características funcionais têm sido pouco aplicadas à culturas e agroecossistemas (Martin & Isaac 2018; Hond-Vaccaro et al. 2023; Dmuchowski et al. 2024), evidenciando a necessidade de avaliação das características funcionais das espécies em SAFs para ajudar na compreensão da dinâmica e funcionamento desses sistemas.

1.2 Características Funcionais

Historicamente, os estudos correlacionam de forma positiva o funcionamento e a provisão de serviços ecossistêmicos com a riqueza de espécies de uma comunidade (Tilman et al. 1997; Cardinale et al. 2007). Contudo, estudos apontam as características das espécies como

parâmetro relevante além da riqueza (Hooper et al. 2005; Rosenfield & Müller 2020). Sendo assim, as características funcionais são definidas como qualquer característica morfológica, fisiológica ou fenológica mensurável ao nível individual, desde a célula até ao nível do organismo inteiro (Violle et al. 2007). Essas características permitem avaliar a relação de aspectos que possam influenciar a biologia de uma espécie, explorando os mecanismos associados à estruturação de comunidades e funcionamento dos ecossistemas (Pavoine & Bonsall 2010). Exemplos desses atributos funcionais, comumente mensurados, são: área foliar, tamanho de raiz, tamanho da semente, altura da planta, capacidade de fixação de nitrogênio, entre outros (Pérez-Harguindeguy et al. 2013).

As plantas apresentam diferentes demandas para a alocação de recursos que são refletidas a partir das características funcionais e determinam as estratégias ecológicas das espécies. Estas estratégias variam desde espécies aquisitivas (com rápida aquisição de nutrientes e renovação foliar) a espécies conservativas (de crescimento lento e alta longevidade foliar) (Wright et al. 2004; Gagliardi et al. 2023). Sendo assim, as características de plantas refletem nas propriedades do ecossistema, na velocidade de assimilação de nutrientes, produtividade, disponibilidade de nutrientes para o sistema e digestibilidade para herbívoros (Shipley et al. 2016; Santos 2020). O equilíbrio entre este contínuo de estratégias em uma comunidade pode ser favorecido em Sistemas Agroflorestais, através do consórcio entre diferentes espécies, o que consolida uma maior variabilidade de processos ecológicos.

Entre os atributos frequentemente mensurados, há um destaque para as características de plantas acima do solo como a variação da área foliar e investimento em carbono para construção das mesmas (área foliar específica e conteúdo de matéria seca foliar) e a relação com as taxas de crescimento e fotossintética (Cornelissen et al. 2003; Wright et al. 2004); renovação foliar à sazonalidade climática (Givnish 1987); a densidade da madeira a funções de suporte, transporte e armazenamento, frequentemente associando um trade-off de compensação e segurança hidráulica, em que baixas densidades parecem indicar um rápido crescimento com um baixo crescimento em volume em relação às altas densidades, que parecem indicar um crescimento lento que reflete a segurança hidráulica, sobrevivência e resistência mecânica (Pérez-Harguindeguy et al. 2013).

Ainda, algumas características funcionais acima do solo frequentemente estudadas, apoiam a sobrevivência e o crescimento das plantas em diferentes condições do ecossistema (Rawat et al. 2021), como a área específica da folha (SLA), a área foliar (LA), o teor de matéria seca da folha (LDMC) e o teor de nitrogênio (NR). Alto SLA e NR são frequentemente associados à altas taxas de crescimento relativo, taxa fotossintética,

manutenção positiva do maquinário fotossintético e facilidade na aquisição de recursos. Ademais, a variação da área foliar é extremamente relacionada à variação das condições de clima, relevo, limitação nutricional, cujo a pressão do ambiente tende a selecionar folhas relativamente menores, além de influenciar nos processos de captação de luz e trocas gasosas da planta. Já o LDMC tende a ser negativamente correlacionado com o SLA e taxa de crescimento relativo, isto porque ele reflete principalmente a quantidade de massa fresca dentro do volume foliar, mas apresenta positiva relação com a longevidade e resistência das folhas. Contudo, poucos estudos exploram esta dinâmica em SAFs (Martins et al. 2023; Santos et al. 2021) e só recentemente estudos vêm buscando avaliar características abaixo do solo (ou radiculares).

As raízes apresentam papel importante na aquisição de água e nutrientes, sobretudo por meio de raízes finas (McCormack & Iversen 2019; Shiha et al. 2022). Assim, os ajustes subterrâneos envolvem a alocação de biomassa para o sistema radicular, a densidade das raízes e comprimento de raízes finas (Hertel et al. 2013; Padilla et al. 2015). Além disso, a mortalidade de raízes finas contribui de 18 a 58% do N total para os solos florestais (Yuan & Chen 2010), tornando a adição de N na serapilheira da raiz maior do que a da serapilheira acima do solo em alguns ecossistemas (Kondori et al. 2021).

Neste contexto, as raízes finas podem ser consideradas uma característica de interesse abaixo do solo. Estas são consideradas como raízes com ≤ 2 mm de diâmetro (King et al. 2021) e representam acima de 90% do comprimento total do sistema radicular (Ratuchne et al. 2016). Além disto, estas raízes são bastante dinâmicas e contribuem significativamente para os ciclos dos nutrientes, influenciando na produtividade primária líquida, além de contribuírem e serem moldadas pela qualidade do solo, impactando na absorção de recursos e interações mutualísticas com a biota do solo (Freschet et al. 2021).

Deste modo, estudos abrangendo as características funcionais através da ecologia de SAFs buscaram integrar a produtividade de forrageiras e culturas anuais, a resposta às pressões por patógenos, relações entre biomassa e nitrogênio, variações anatômicas (Siegwart et al. 2022; Gagliardi et al. 2023). No entanto, no Brasil, os estudos que buscam compreender a relação do funcionamento ecológico dos SAFs, a partir de características funcionais, com a estrutura e desenvolvimento das espécies vegetais (Martins et al. 2023), supressão de ervas espontâneas e proteção do solo (Santos et al. 2021), incremento de funções ecossistêmicas (Blesh 2018), ciclagem de nutrientes em grupos sucessionais (Steinfeld et al. 2024) são recentes.

1.3 Diversidade Funcional

A demanda por explicar as relações na variação das características funcionais de plantas com questões ecológicas e associações biológicas, em diversas escalas, deu origem a formas padronizadas de medir a diversidade funcional de diferentes níveis organizacionais (Villéger et al. 2008; Laliberté & Legendre 2010). A diversidade funcional é amplamente atribuída à distribuição da gama de características funcionais em um sistema (Tilman et al. 1997), ou as diferenças multivariadas das características dentro de uma comunidade (Cadotte et al. 2011) e vem sendo utilizada para descrever diferentes aspectos da estrutura de comunidade em relação às características, podendo ser melhor preditora que as métricas de diversidade baseadas apenas na riqueza de espécies (Petchey & Gaston 2002; Mason et al. 2005; Isbel et al. 2017).

Os modelos estatísticos normalmente assumem que a diversidade funcional equivale à complementaridade das espécies no uso de recursos, ou seja, que as diferenças na forma como as espécies obtêm e usam os recursos são representadas pela diversidade funcional (Tilman et al. 1997; Loreau 1998). Sendo assim, esses mecanismos relacionados à utilização de recursos a partir de maior diversidade de estratégias, leva a uma utilização mais eficiente dos recursos e, conseqüentemente, a uma maior produtividade (Naeem et al. 1994; Loreau 1998). Assim, os mecanismos propostos que inferem uma ligação entre a complementaridade de nicho com a diversidade e função do ecossistema, baseiam-se na distribuição das espécies em um nicho espacial (Mason et al. 2005).

O poder explicativo e preditivo da diversidade funcional é provavelmente maior quando são utilizadas diferentes características relacionadas a aquisição e uso de recursos entre espécies e em escalas espaciais relativamente locais. Assim, buscando maximizar o poder explicativo da diversidade funcional, selecionar características é uma etapa importante, buscando descrever as funções da melhor forma possível e evitando redundâncias (Villéger et al. 2008), sobretudo em sistemas dinâmicos como os Agroflorestais.

Dentre as métricas que mais descrevem a distribuição de características num espaço, as que mais se destacam nos últimos anos são: Riqueza funcional (*Fric*), dispersão funcional (*Fdis*), Redundância funcional (*Fred*), Divergência funcional (*Fdiv*), Equabilidade funcional (*FEve*) e mais recentemente os modelos de hipervolume probabilístico (HV) (Petchey & Gaston 2002; Carmona et al. 2016; Blonder 2018).

A riqueza funcional (*Fric*) reflete a quantidade de espaço de nicho ocupado pelas espécies dentro de uma comunidade. Elevados valores de riqueza funcional indicam que os

recursos potencialmente disponíveis à comunidade estão sendo usados, refletindo diretamente na complementaridade de nicho, sendo esta métrica definida como a diferença entre a diversidade de espécies e a diversidade funcional (Equação I).

$$FR = D - Q$$

Em que, D é o índice de Gini-Simpson para diversidade de espécies, cujo p_i é a proporção da abundância de espécies i em uma comunidade com um total de s espécies e Q é a entropia quadrática de Rao, considerada uma medida apropriada de Diversidade Funcional (Dukát 2005; Mason et al. 2005; Santos 2020).

A uniformidade funcional (FEve) quantifica a regularidade com que o espaço funcional é preenchido pelas espécies, ponderado pela abundância das características. A uniformidade funcional pode ser vista como o grau em que a biomassa de uma comunidade é distribuída no espaço de nicho para permitir a utilização eficaz de toda a gama de recursos disponíveis (Mason et al. 2005). Esta métrica funcional diminui quando a abundância é distribuída de maneira menos uniforme entre as espécies ou quando as distâncias funcionais entre as espécies são menos regulares, sendo uma medida que não é influenciada pela riqueza de espécies e restrita entre 0 e 1 (Villéger et al. 2008). A expressão matemática (Equação II) que descreve FEve indica que PEW_i é equidade parcial ponderada e o termo $1/(S - 1)$ é subtraído do numerador e do denominador porque existe pelo menos um valor de PEW que é menor ou igual a $1/(S - 1)$ seja qual for S (Villéger et al., 2008).

$$FEve = \frac{\sum_{i=1}^{S=1} \min \left(PEW_i, \frac{1}{S-1} \right) - \frac{1}{S-1}}{1 - \frac{1}{S-1}}$$

A dispersão funcional (Fdis) representa como a abundância é espalhada ao longo de um eixo de característica funcional, dentro da faixa ocupada pela comunidade (Mason et al. 2005), reduzindo o valor conforme espécies mais abundantes aproximem suas características funcionais ao centro da faixa de características. Num contexto multivariado, a dispersão funcional (Equação III) está relacionada com a forma como a abundância é distribuída dentro do volume do espaço de características funcionais ocupado pelas espécies, podendo indicar alta diferenciação de nichos e então baixa competição por recursos (Mason et al. 2005; Villéger et al. 2008; Santos 2020), podendo ser calculada por:

$$FDiv = \frac{\Delta d + \overline{dG}}{\Delta|d| + \overline{dG}}$$

Os valores de dG_i são distâncias euclidianas, enquanto Δd é limitado entre $\overline{dG}$ e $\Delta|d|$ e $|$. Portanto, a adição de $\overline{dG}$ ao numerador e ao denominador garante que o índice varie entre

0 e 1. O índice se aproxima de 0 quando espécies altamente abundantes estão muito próximas do centro de gravidade em relação às espécies raras e aproxima-se da unidade quando espécies altamente abundantes estão muito distantes do centro de gravidade em relação às espécies raras (Villéger et al. 2008).

O hipervolume n-dimensional foi originalmente proposto por Hutchinson em 1957 para descrever o nicho fundamental de uma espécie. Esses hipervolumes existem dentro de um espaço definido por um conjunto de n eixos independentes. Este subconjunto do espaço n-dimensional é o hipervolume definido de forma. Assim, os hipervolumes podem ser definidos usando muitos tipos de eixos, como características funcionais, clima, recursos; em muitos sistemas como indivíduos, populações, espécies, comunidades (Blonder 2018).

Esta função é considerada como uma função de densidade de probabilidade e, quando se há um intervalo contínuo de valores, existem muitos valores possíveis sob uma função de distribuição de probabilidade arbitrária. Como resultado, existem vários limites, volumes e formas possíveis que dependem do intervalo de dados, cujo a interpretação permite variação na probabilidade de pontos dentro do hipervolume.

Na ecologia funcional algumas aplicações à estrutura da comunidade foram desenvolvidas buscando entender como espécies de nichos fundamentais muito próximos não poderiam coexistir, além de inferir que hipervolumes muito grandes indicam processos competitivos. Este modelo tem sido usado para testar gradientes funcionais no uso da terra, sucessão ecológica, queimadas e resumir a ocupação e sobreposição de espaços de estratégia ecológica (Blonder 2018). Assim, estudos envolvendo as características e diversidade funcional vêm sugerindo uma correlação positiva entre maximização da estrutura e diversidade funcional com a multifuncionalidade de agroecossistemas (Wood et al. 2015; Finney & Kaye 2017).

2. Age-driven shifts in functional composition and diversity shape ecosystem service provision in agroforestry systems

2.1 INTRODUCTION

The conversion of tropical forests into alternative land uses has led to a socio-environmental crisis, undermining ecological sustainability and compromising essential ecosystem services, such as nutrient cycling and carbon storage (Lapola et al. 2023). In this context, agroforestry systems (AFS) have been promoted as sustainable alternatives to deforestation and conventional agriculture, particularly in tropical regions such as the Brazilian Amazon (Kouassi et al. 2024). AFSs reconcile agricultural production with the maintenance of ecosystem services, including climate regulation, carbon sequestration, and soil restoration and conservation by integrating trees into agricultural landscapes (Dmuchowski et al. 2024). Moreover, AFSs represent a promising strategy for climate change mitigation and food security (Isaac & Borden, 2020).

The configuration of AFS is largely shaped by local knowledge, production goals, farmers' species preferences, and management strategies, resulting in diverse vegetation structures with different ecological functions (Vineeta et al. 2022). Although species recommendation lists have been developed based on farmers' preferences, most of these approaches remain taxonomic, emphasizing species identity rather than their ecological and ecosystem functions (Isaac et al. 2024). As AFSs age, they undergo significant changes in ecological functions, productivity, and soil properties (Kinyili et al. 2024). Thus, it is crucial to understand how these systems develop over time and how their ecosystem functions stabilize within managed landscapes. Several ecosystem functions, such as aboveground biomass accumulation, litter input, and canopy stratification, tend to increase with AFS age, contributing to improved nutrient cycling and microclimatic regulation (Vineeta et al. 2022; Gou et al. 2023). Besides, age plays a key role in this context, as it is also influenced by management practices that jointly shape AFSs characteristics. For instance, management practices such as crop fertilization, and livestock grazing influence soil physical and chemical properties through nutrient addition or, conversely, nutrient mining and soil compaction (Noulékoun et al. 2023). Although AFSs age can be used as a proxy for successional trajectories, the functional structure of these systems emerges not only from environmental filters associated with time, but also from management practices. Farmers often remove short-lived species and introduce long-lived or economically relevant species at specific stages or apply regenerative agricultural practices such as crop rotation (Lopes Costa et al. 2024), which may interact with environmental filtering

to shape functional identities and diversity. Therefore, investigating the role of age in shaping ecosystem functions provides key insights into the ecological sustainability of these systems in the Amazon region.

Advances in using functional traits (i.e. morphological, physiological, and phenological features that influence plant performance) to understand resource-use strategies, ecosystem functioning (Violle et al. 2007; Isaac et al. 2024), and functional diversity (e.g. the variation of species' traits within a community) had emerged as a key indicator of ecosystem functionality (Santos et al. 2021). However, critical gaps remain regarding how these traits vary with AFS age. Few studies assess traits of both above and belowground organs or investigate how these traits coordinate to influence multiple ecosystem functions (Bergmann et al. 2020, La Bella et al. 2024). Moreover, the application of the leaf economics spectrum as an important explanatory axis for functional variation which influences ecosystem properties, such as nutrient assimilation rates, availability, and overall productivity (Shipley et al. 2016), is still underexplored in tropical agroforestry contexts. There is also a substantial knowledge gap regarding its validity for root traits, which may also reflect conservative-acquisitive strategies (Reich, 2014). The lack of integrative approaches linking above and belowground traits in Amazonian AFSs settings represents a major limitation for function-oriented system design.

In this context, the acquisitive-conservative axis, extensively explored in age-driven natural ecosystems (Díaz et al. 2015; Lohbeck et al. 2013; Botelho et al. 2025), acquires a new layer of complexity in agroforestry systems. In these managed environments, functional strategies do not emerge solely from ecological filters, such as canopy closure or increased soil fertility (Martius et al. 2004), but are continuously reshaped by human intervention. The deliberate selection of economically valuable species, the removal of target trees, the intensification of grazing, or the alteration of soil fertility through fertilization may interrupt the expected successional trajectory and redirect resource-use strategies (Jiang et al. 2023; An et al. 2025). As a result, ecological patterns traditionally constrained by evolutionary and environmental pressures may become flexible, oscillating between acquisitive and conservative extremes across different management stages. This suggests that functional identity in agroforestry may be not only a reflection of natural gradients, but also a product of cultural and economic decisions, raising fundamental questions about how human agency mediates ecological strategies and the long-term functioning of managed ecosystems (Oliveira et al. 2025). Thus, AFS age serves as a proxy that integrates both management-driven filtering at early stages and environmentally driven filtering at later stages.

Another critical gap is related to the growing interest in ecosystem services as a way to

recognize the value of ecosystems and encourage their sustainable use (Millennium Ecosystem Assessment, 2005), which has drawn attention to how different organisms support the delivery of these services (Suding et al. 2008). In this context, a key challenge lies in understanding how functional traits and diversity influence ecosystem effective multifunctionality, defined as the capacity of ecosystems to sustain multiple functions simultaneously. In AFS, functional diversity is particularly relevant (Santos et al. 2021), as species combinations with different ecological strategies can optimize the provision of multiple ecosystem services, such as nutrient cycling, maintenance of soil fertility, biomass accumulation, and microclimatic regulation (Gou et al. 2022). In this context, while previous studies in other regions have shown multiple benefits of AFSs (Veldkamp et al. 2023; Satish et al. 2024) and effects of stand age (Kinyili et al. 2024), we quantify effective multifunctionality in Amazonian AFSs, capturing the simultaneous performance of multiple ecosystem functions across age gradients. To date, and to the best of our knowledge, no research on Amazon has investigated AFS multifunctionality from a functional trait perspective across systems of varying ages and ecological strategies. This approach allows us to test whether older AFSs, having undergone both early management filtering and subsequent environmental assembly, exhibit higher levels of multifunctionality than younger systems.

Thus, this study aims to fill these gaps by integrating aboveground (leaf and wood) and belowground (root) functional traits to explore how plant ecological strategies and functional diversity shape ecosystem multifunctionality across an age-gradient of AFS in the Western Amazon. We highlight the integration of above and below ground functional traits to understand how different AFSs structures over time influence multiple ecosystem functions, framed within the plant economic spectrum (acquisitive-conservative axis). Furthermore, this study provides scientific support for public policies that promote sustainable land use in the Amazon, such as “Brazil’s Política Nacional sobre Mudança do Clima (Lei 12.187/09)” law, which promotes greenhouse gas emission mitigation targets and low-carbon agriculture. At a broader level, the study also contributes to global commitments like the UN Sustainable Development Goals (SDGs), especially SDG 2 and 13 (zero hunger, sustainable agriculture, and climate action) (United Nations, 2015). Thus, our results offer locally grounded, yet potentially generalizable insights, suggesting that higher functional diversity in older AFSs may contribute to sustainable development and climate mitigation efforts.

Our study investigates the functional relationships among plant species in AFS, their ecological strategies, and multiple ecosystem functions in AFSs of different ages in Western Amazonia. Specifically, we aimed: (i) to analyze ecological strategies and functional diversity

of plants in AFSs along an age gradient, evaluating leaf, stem, and root functional traits; (ii) to verify whether AFS age enhances ecosystem functions and multifunctionality; (iii) to evaluate the influence of functional identity and diversity on ecosystem functioning using proxies of different ecosystem functions and the effective multifunctionality; (iv) to investigate the degree of coordination between above- and belowground trait functional variation axes within AFS.

We hypothesized that (i) older AFSs would tend to exhibit more conservative functional strategies related to resource use (higher leaf dry mass content - LDMC, wood density - WD, root tissue density - RTD, and root diameter - RD), due to increasing canopy closure as the AFSs develop, but that these patterns are mainly modulated by early management interventions (Figure 1A); (ii) we expected a multifunctionality transition, with younger AFSs exhibiting lower value of multifunctionality compared to more mature systems (Figure 1B). Furthermore, (iii) we expect that functional identity, reflecting the dominant species selected by farmers, will have a stronger influence on the provision of ecosystem functions, since management decisions determine which species persist and which are removed. In this context, the functional identity of dominant species is likely to drive system-level processes more than the overall trait variation within the community; and (iv) we hypothesized that above- and belowground functional traits will reveal coordinated patterns in AFSs, although partial decoupling may occur due to distinct environmental and management-driven filters.

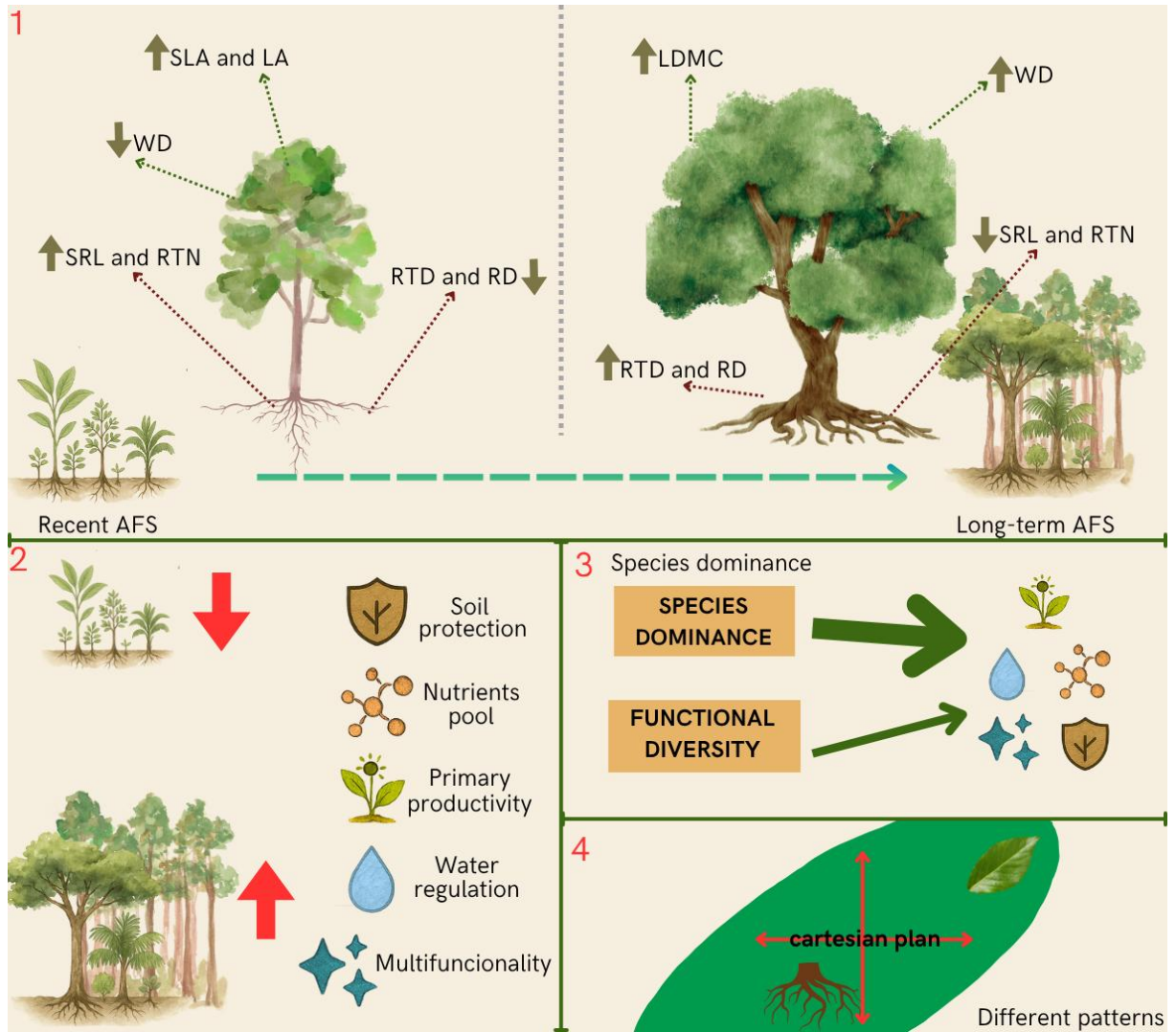


Figure 1. We expected (1) long-term AFS to exhibit a more conservative ecological strategy (dashed green arrow), with higher leaf dry mass content (LDMC) values along the leaf economic spectrum. In contrast, younger AFS would display a more acquisitive strategy, characterized by higher specific leaf area (SLA) values, due to increasing canopy closure as the AFSs develop. However, these patterns are mainly modulated by early management interventions; (2) we further expected that older AFSs would exhibit higher ecosystem multifunctionality, driven by the accumulation of attributes supporting a broader range of ecosystem functions (red arrows); and (3) that functional identity (species dominance) will have a stronger influence (thick green arrow) on the provision of ecosystem functions than functional diversity (thin green arrow). Management decisions determine which species persist and which are removed, thereby shaping both identity and diversity; Finally, we expect that (4) the coordination of other traits associated with conservative plant strategies, such as higher root tissue density (RTD), root diameter (RD), and wood density (WD) in older AFS, whereas traits linked to acquisitive strategies, such as higher specific root length (SRL) and root tip abundance (RT), would be more prominent in younger AFS.

3 MATERIALS AND METHODS

3.1 Study Area

We conducted this study in agroforestry systems located in Seringal Porongaba, Seringal Povir and Seringal Filipinas, within the Chico Mendes Extractive Reserve region, Acre, Brazil (Figure 2).

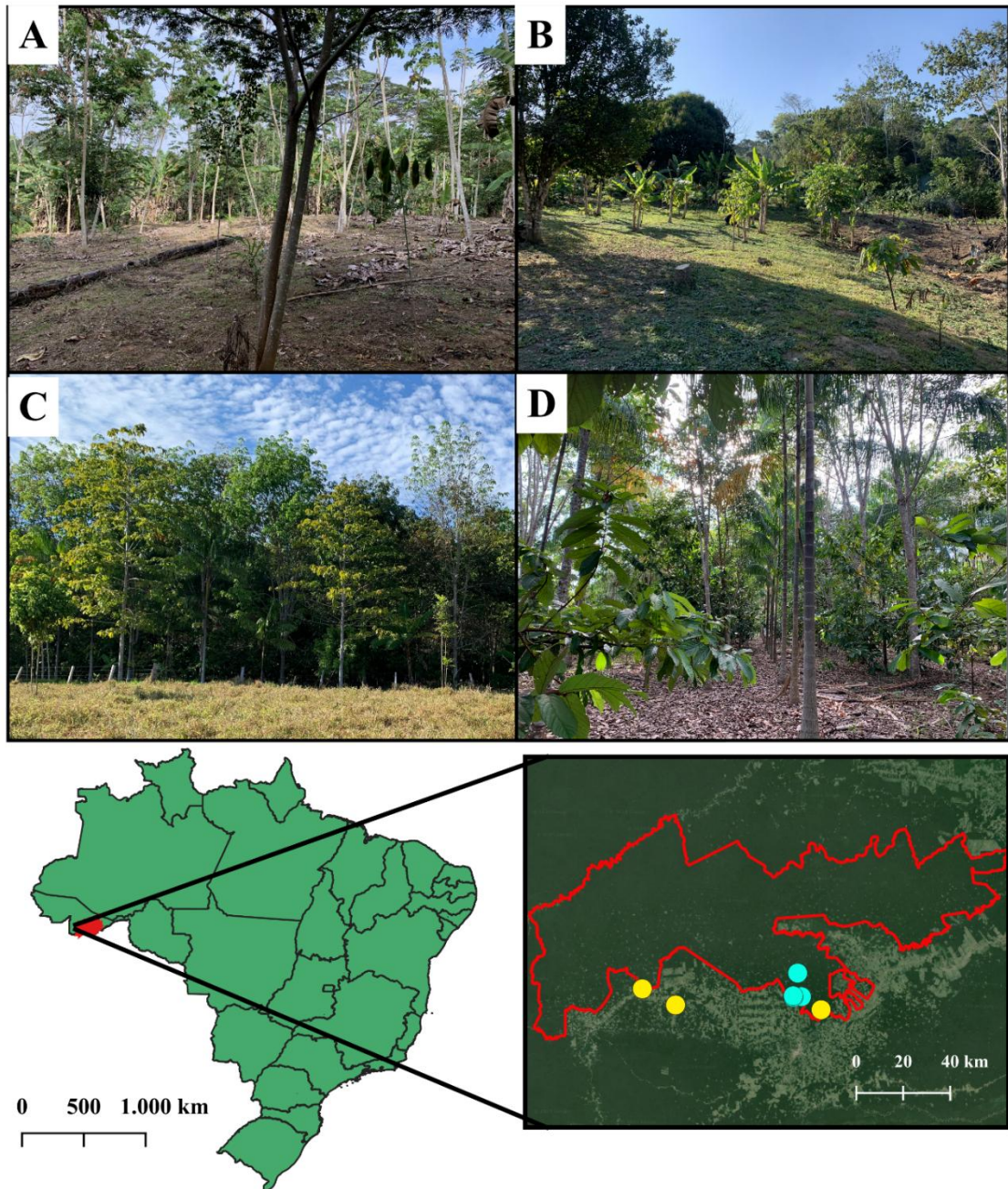


Figure 2. Study area map showing the location of the eight (8) agroforestry systems evaluated. Each system includes five (5) plots, represented by individual points on the map. Long-term systems ($n = 4$) are marked in yellow, and recent ones ($n = 4$) in blue. The boundary marked in red represents the Chico Mendes Extractive Reserve, in Acre. Images A and B depict recent AFSs, while C and D show long-term AFSs.

The predominant soil types in the area are Red-Yellow Podzols, Eutrophic Red-Yellow Podzols, and Eutrophic Gleyed Hydromorphic soils. The climate is classified as tropical monsoon (AM) according to the Köppen system, with a mean annual temperature ranging from 26°C to 27°C and a short dry season. The region has a marked rainy season from November to April and a drier period from June to August, while annual precipitation ranges from 1,800 mm to 2,200 mm (Brasil 2006; Reichle et al. 2022). The research, biological sampling, plant material collection, and transport were authorized by the “Sistema de Autorização e Informação em Biodiversidade” (SISBIO, license number 86402-1).

These AFSs are managed by traditional extractivist families, often composed of smallholders, whose livelihoods combine forest product extraction (such as Açaí - fruits of *Euterpe oleracea* - and Brazil nuts) with subsistence agriculture and, to a lesser extent, local market trade. The adoption of AFSs in these areas has been promoted as a strategy to combine forest conservation with income generation, since they are also part of a forest restoration research initiative.

3.2 Sampling Design

We selected eight (8) AFSs ranging in age from 1 to 20 years (recent AFSs: 1-2 years; long-term AFSs: 6-20 years). These AFSs were chosen within this age range because they are part of a forest restoration research initiative under the same funded project, and they were the AFSs available in the Chico Mendes Extractive Reserve (Table 1).

Table 1. Characterization of the evaluated Agroforestry Systems. The table summarizes the main features of each system, including their agricultural purpose.

AFS	Category	Age (years)	Area (m ²)	Soil preparation	Fertilization	Main species / Observations	Mean litter (g)
1	Young	2	6,759.05	Harrowing	Once before planting	<i>Mangifera indica</i> (pre-existing); no chemical fertilization	219.29
2	Young	1	3,765.45	Harrowing	None	Recently planted <i>Euterpe oleracea</i> (not measured); presence of <i>Ananas comosus</i>	115.90
3	Old	10	9,356.82	Harrowing	Once before planting	<i>Musa sp.</i> initially planted to provide shading for slow-growing species such as <i>Hevea brasiliensis</i> , and <i>Bertholletia excelsa</i>	239.04

4	Old	17	7,473 .79	None	None	<i>Theobroma grandiflora</i> , <i>Persea americana</i> ; <i>Coffee sp.</i> no longer managed; two cropping modules	314.46
5	Young	1.1	8,908 .68	Not mentioned	Green manure with <i>Arachis pinto</i>	<i>Mangifera indica</i> (older individuals), <i>Musa sp.</i> in separate module; subsistence use	26.69
6	Young	1.5	3,308 .15	Partial (in one section)	Yes, partially	Focus on <i>Musa sp.</i> ; <i>Mangifera indica</i> (older individuals); close to the smallholder's residence	216.92
8	Old	20	11,32 8.98	None	None	<i>Musa sp.</i> and <i>Citrus sp.</i> initially (later removed); near of a pasture	662.98
9	Old	6	34,98 0.41	Harrowing; initial maize cultivation	Not informed	<i>Coffea sp</i> as main crop; maize used for green cover	564.94

In each AFS, we installed five (5) random plots of 100 m² (10x10m) to assess species composition and plant functional traits. The criteria for individuals' inclusion were the circumference at breast height (CBH) >10 cm. The threshold of 10 cm CBH (equivalent to approximately 3.18 cm DBH) was chosen to include a greater number of species, especially in younger AFSs, which tend to be composed of smaller individuals (Figure 3).

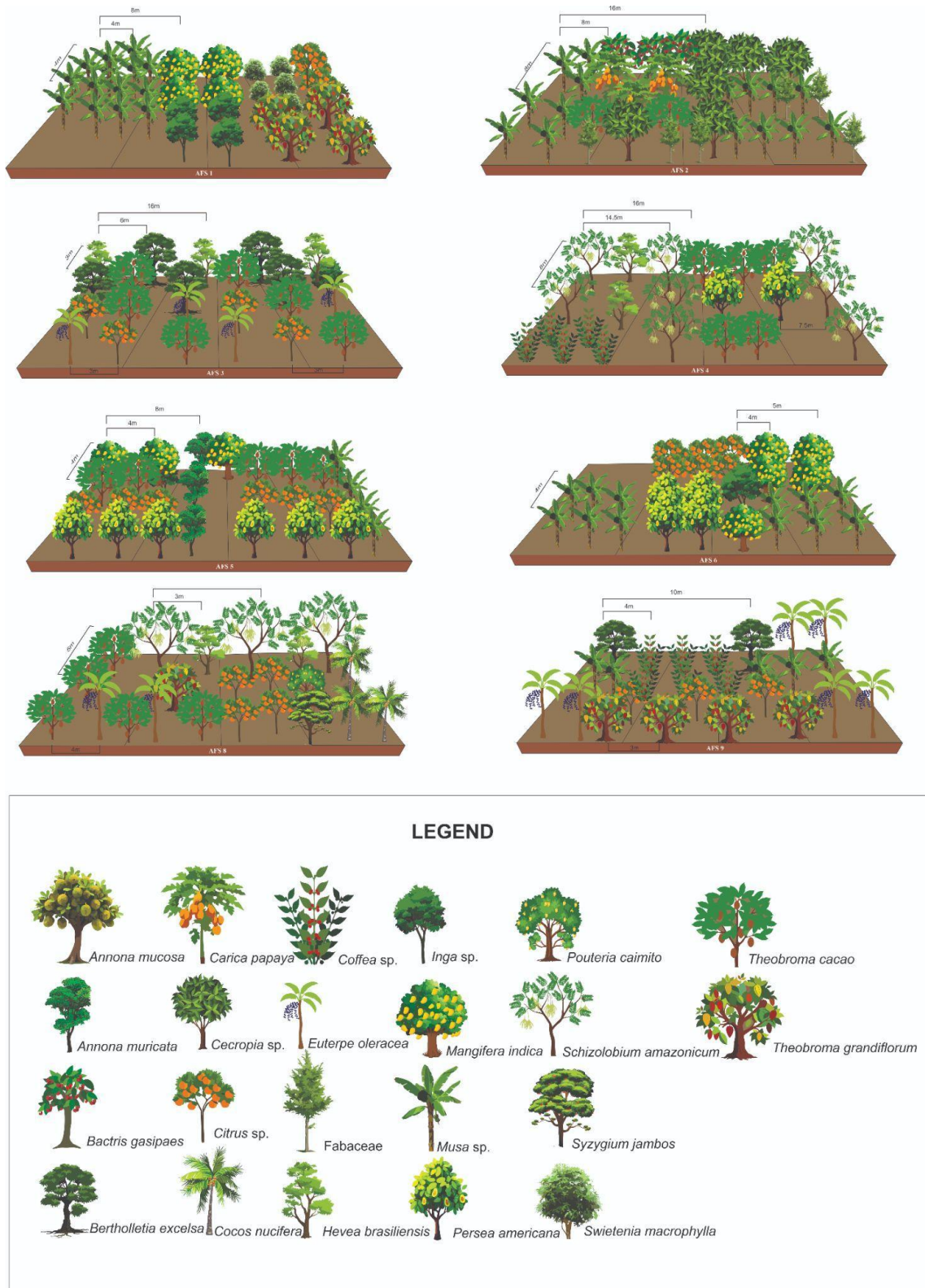


Figure 3. Spatial arrangement of plants in each AFS. Density is expressed as stems ha^{-1} rather than individuals ha^{-1} , since species such as *Musa sp.* produce multiple shoots per individual, which more accurately reflects the structure and space occupation in the system. Stem densities (mean $\pm$ SD) were: AFS 1: 1540 ± 691 ; AFS 2: 1660 ± 688 ; AFS 3: 1360 ± 808 ; AFS 4: 880 ± 249 ; AFS 5: 640 ± 321 ; AFS 6: 2200 ± 1334 ; AFS 8: 1400 ± 612 ; AFS 9: 1180 ± 179 .

The ecosystem functioning measurements were carried out in a subset of three plots per AFS, in the center of the 10x10 plot, selected to represent the overall conditions of the system. Functional traits were measured on all plant species with 3-5 individuals per AFS, totalizing 15 species with functional traits measured (Table 2).

Table 2 - List of species with functional traits evaluated in Agroforestry Systems.

AFS	Age	Species	Mean branch diameter
1	2 years	<i>Musa sp</i>	NA
1	2 years	<i>Mangifera indica</i>	2.762
1	2 years	<i>Citrus sp</i>	6.432
1	2 years	<i>Swietenia macrophylla</i>	5.506
2	1 years	<i>Cecropia sp</i>	21.297
2	1 years	<i>Musa sp</i>	NA
2	1 years	<i>Parkia nitida</i>	3.115
3	10 years	<i>Theobroma grandiflorum</i>	5.742
3	10 years	<i>Hevea brasiliensis</i>	7.353
3	10 years	<i>Euterpe oleraceae</i>	NA
3	10 years	<i>Annona muricata</i>	3.092
4	17 years	<i>Theobroma grandiflorum</i>	4.834
4	17 years	<i>Coffea sp.</i>	5.64
4	17 years	<i>Persea americana</i>	5.08
4	17 years	<i>Schizolobium amazonicum</i>	4.8
5	1.01 year	<i>Theobroma grandiflorum</i>	4.415
5	1.01 year	<i>Citrus sp</i>	4.257
5	1.01 year	<i>Mangifera indica</i>	5.605
5	1.01 year	<i>Persea americana</i>	5.461
6	1.5 year	<i>Inga sp</i>	4.701
6	1.5 year	<i>Citrus sp.</i>	3.526
6	1.5 year	<i>Mangifera indica</i>	5.207
6	1.5 year	<i>Persea americana</i>	5.447
8	20 years	<i>Theobroma grandiflorum</i>	3.811

8	20 years	<i>Euterpe oleraceae</i>	NA
9	6 years	<i>Theobroma cacao</i>	3.897
9	6 years	<i>Coffea sp.</i>	3.804
9	6 years	<i>Citrus sp.</i>	3.576
9	6 years	<i>Musa sp.</i>	NA
9	6 years	<i>Euterpe oleraceae</i>	NA

This criterion was also defined by logistical and time constraints, while also in some AFSs rarer species often occurred with only one, or rarely two individuals. We prioritized reproductively mature and healthy-looking individuals whenever possible (Pérez-Harguindeguy et al. 2013). The threshold of 10 cm CBH (equivalent to approximately 3.18 cm DBH) was chosen to include a greater number of species, especially in younger AFSs, which tend to be composed of smaller individuals.

3.3 Plant traits measurement

We measured a set of functional traits across various plant organs (root, stem, and leaf), aiming to capture an integrated view of plant strategies. Trait measurements followed the standardized protocol proposed by Pérez-Harguindeguy et al. (2013). We selected ten (10) traits from both above - and belowground organs: plant height (H), leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), wood density (WD), specific root length (SRL), root tissue density (RTD), specific root area (SRA), mean root diameter (RD), and number of root tips (RT). To measure each trait:

Aboveground Trait – Tree Height

Tree height was measured using the Arboreal Tree application (Pace et al. 2022) on an iPhone 12 Pro. which incorporates a LiDAR scanner within the camera system. allowing for object measurement and spatial depth mapping. The app calculates tree height using trigonometric principles. Prior to field use. it was tested on trees of known height. showing a measurement deviation of approximately 15 cm. According to the manufacturer. the measurements provided by the app are highly accurate and comparable to those obtained using professional forestry instruments such as the Haglöf Vertex II (Arboreal AB. Umeå. Sweden).

Aboveground Traits – Leaf Traits

Ten fully expanded leaves were selected per individual, avoiding those with signs of pathogen or herbivore damage. Sampling prioritized sun-exposed leaves by collecting intact branch sections with the leaves still attached, which were only removed shortly before processing. Branches were stored with their base wrapped in moist paper and placed in sealed, dark plastic bags to maintain high humidity and minimize transpirational water loss (Pérez-Harguindeguy et al., 2013).

In the laboratory, leaf area (mm^2) was measured by scanning the leaves individually and analyzing the images in R using the EImage package (Maracahipes-Santos et al. 2023). For both simple and compound leaves the petiole was excluded, and in the case of compound leaves the total leaf area was calculated as the sum of all leaflets and we didn't consider the wood veneer rack. Specifically to the Acai palm, we divided it into three sections (bottom, mid and top) since the form of the Acai palm seems like an ellipse. Then, we measured the leaf area for the Acai's palm to ten leaflet and estimated the value for the total number of leaflets in each section, then we sum the value of the three sections. Thus, for *Musa sp.*, we measured the length and width of the third leaf. We then estimated the total leaf area using the equation from Zucoloto et al. (2008), which incorporates the number of total leaves: $LA = 0.5187 * (\text{leaf length} * \text{leaf width} * \text{number of total leaves}) + 9603.5$.

To calculate specific leaf area (SLA) and leaf dry matter content (LDMC), the leaves were saturated with water, weighed fresh, then oven-dried at 65°C for 72 hours and weighed again using a digital balance with 0.001 g precision. Branch samples used for wood density determination were taken from sun-exposed outer canopy branches. Trait values were calculated using the following equations:

$$\text{Equation I. SLA} = (\text{leaf area})/(\text{dry mass}) (\text{mm}^2)/(\text{g})$$

$$\text{Equation II. LDMC} = (\text{dry mass})/(\text{saturated fresh mass}) (\text{mg})/(\text{g})$$

Aboveground Traits – Stem Trait

To determine wood density (WD) for most plant species, 60 cm long branches were collected from the tip to standardize sampling. The lower 30 cm of each branch was divided into two segments (5 cm above and below the midpoint). WD was calculated based on the displaced volume of each segment using Archimedes' principle: each segment was submerged in a container of water placed on a balance, and the increase in mass (g) was used to determine the displaced volume (cm^3). The segments were then oven-dried at 65°C for 72 hours to determine dry mass. WD was calculated as:

Equation III. $WD = (\text{dry mass})/(\text{displaced volume}) \text{ (g)/(cm}^3\text{)}$

Specifically, to *Euterpe oleraceae* (Açaí palm), because this species was consistently sampled, and its stem architecture does not allow branch sampling, WD values were obtained from the literature (Cardoso-Junior et al. 2017) and applied uniformly across all AFS. Thus, as *Musa sp.* (Banana) is a perennial herb with a pseudostem, WD cannot be estimated using the branch-segment method. Instead, we used published WD values estimated specifically for banana pseudostems (Guimarães et al. 2014).

Belowground Traits – Root Traits

Fine roots were sampled down to 30 cm soil depth using a destructive but low-impact tracing method. Starting at the base of the stem, coarse roots were carefully exposed with a hand spade and followed into the soil. From these, smaller root branches were traced and excavated with a brush until reaching fine roots of approximately 0.2 mm in diameter. This procedure ensured that the sampled roots were unequivocally associated with the targeted individual, avoiding the collection of roots from neighboring trees or crops within the AFS.

After collection, roots were washed and stored at 6°C in a refrigerator, following the protocol by Freschet (2020). In the lab, roots were washed again to remove soil residues and then prepared for morphological analysis. They were immersed in water and scanned at 400 dpi using a flatbed scanner. Root images were processed in RhizoVision to estimate the traits: Specific root length (SRL), average root diameter (RD), specific root area (SRA), root tissue density (RTD) and number of root tips (RN), following protocols by Pérez-Harguindeguy et al. (2013), Borden et al. (2020), and Seethepalli et al. (2021). After scanning, the roots were oven-dried at 65°C for 72 hours. The traits were calculated using the following equations:

Equation IV. $SRL = (\text{root length})/(\text{root dry mass}), \text{ (mm/g)}$

Equation V. $SRA = (\text{total root area})/(\text{dry mass}), \text{ (mm}^2\text{/g)}$

Equation VI. $RTD = (\text{dry mass})/(\text{root volume}), \text{ (g/cm}^3\text{)}$

These traits are associated with functional plant strategies and ecosystem functioning (Violle et al. 2007; Pérez-Harguindeguy et al. 2013; Table 3).

Table 3. Measured functional traits and their ecological implications. The "Effect" column indicates the relationship between trait values and their associated ecological outcomes. An upward arrow (↑) indicates that higher trait values are associated with an increase in the corresponding effect.

Trait	Effect	Literature	Prediction: long-term AFS
Plant Height (H. m)	↑Tolerance or resistance to disturbance. the ability to compete for light	Lavorel and Garnier. 2002; Pérez-Harguindeguy et al. 2013	Trees allocate more resources to height growth due to stronger competition for light beneath a closed canopy.
Leaf Area (LA. mm ²)	↑Productivity. nutrient mobilization. disturbance response	Wright et al. 2017	Trees tend to show lower leaf area due to reduced light availability resulting from canopy closure.
Specific leaf area (SLA. mm ² g ⁻¹)	↑Photosynthetic rate. nutrient cycling through litter. and resource uptake efficiency	Wright et al. 2004; Díaz et al. 2016	Trees exhibit lower SLA. reflecting a conservative strategy under reduced light conditions in mature stands.
Leaf dry matter content (LDMC. mg g ⁻¹)	↓Decomposition and nutrient release; ↑Leaf longevity. resistance to physical damage. and herbivory	Pérez-Harguindeguy et al. 2013; Hodgson et al. 2011	Higher LDMC in long-term AFSs. indicating a conservative strategy adapted to low-light available. with increased resistance to stress.
Wood density (WD. g cm ³ ⁻¹)	↑Hydraulic safety. mechanical stability. structural support. and resistance to physical damage	Cornelissen et al. 2003; Chave et al. 2009	Higher WD. due to biomass accumulation and the need for greater structural support in mature individuals.
Specific root length (SRL. mm g ⁻¹)	↑Soil exploration and nutrient/water uptake	Larcher. 2003; Ostonen et al. 2007	Lower SRL. which suggests reduced investment in fine absorptive roots and a shift toward more conservative root systems.
Root tissue density (RTD. g cm ³ ⁻¹)	↓Decomposition and mycorrhizal colonization; ↑Carbon and nutrient retention	Wright and Westoby. 1999; Hernández et al. 2010	Higher RTD. where roots are denser and longer-lived. supporting stability and nutrient conservation.
Specific root area (SRA. mm ² g ⁻¹)	↑Soil exploration for nutrient/water acquisition	Wasaya et al. 2018; Fuchs et al. 2020	Trees showing lower SRA. reflecting lower investment in exploratory root surfaces in stabilized systems.

Root diameter (RD. mm)	↑Carbon storage; ↓Potential for mycorrhizal colonization	Comas & Eissenstat. 2004	Trees have greater root diameter. resulting from long-term carbon investment.
Number of root tips (RT. count)	↑ Soil exploration. water and nutrient uptake. root exudation	Comas & Eissenstat. 2004 ; Freschet et al. 2021	Fewer root tips. associated with reduced investment in exploratory root in stabilized systems.

3.4 Ecosystem function variables

Alongside the floristic inventory, in three of the five 10x10m plots, we installed a 1x1 m subplot (from here on we refer only to it as subplot) to conduct a soil sampling around the center of three plots of each AFS, which resulted on 24 unit samples of ecosystem functions.

Soil samples were collected within a 1x1 m² quadrat placed at the center of the 10x10 m plot. In each plot, at a depth of 0–20 cm, we measured in the first, third, and fifth 10x10 plots of each agroforestry system. The samples were stored and transported to the laboratory. Afterwards, 0.5 kg of soil was separated and air-dried for one and a half weeks. Soil analyses were performed in a specialized laboratory (Soil Department, Agricultural Sciences Center – Federal University of Viçosa) to determine nine (9) variables related to ecosystem functions (Garland et al. 2021; La Bella et al. 2024). Specifically, we analyzed soil electrical conductivity (EC) and water holding capacity (WHC%) as proxies for soil water regulation; total concentrations of nitrogen, phosphorus, calcium, zinc, and remaining phosphorus as proxies for macro- and micronutrient pools; we also evaluated canopy openness and litter dry mass as proxies for soil protection, and the biomass of the evaluated species as a proxy for aboveground primary productivity.

EC was measured using a saturated soil extract with a conductivity meter (1:5 weight/volume extract), and WHC% was determined gravimetrically as the percentage of water retained in the soil at -10 kPa (Ribeiro et al., 1999; Teixeira et al., 2017). Soil nitrogen content was determined through sulfuric digestion and titrimetric quantification following the Kjeldahl protocol; nutrients were determined using the Mehlich 1 extraction method and 1 mol/L KCl extractor for calcium and magnesium (Teixeira et al., 2017).

Leaf litter was collected in the whole 1 m² quadrat in the center of previously mentioned three (3) plots, weighed fresh, and then oven-dried at 65°C for 72 hours to determine litter biomass (Adapted from Liu et al. 2022). Canopy openness was assessed from photographs taken in the field between 6:00 and 7:00 AM (with diffuse light). Here we argue that the equipment model - professional camera or cell phone - is not so important since we do not

specifically use the resolution to estimate the canopy opening, but only the image contrast. 3 photos for each subplot were taken at breast height, with the camera facing upward and at a 0-degree inclination. The images (959 x 1280 pixels / 96 dpi) were binarized in R software, where images were converted to grayscale and then binarized using a fixed threshold value of 0.5 (on a 0-1 scale), where pixels darker than the threshold were classified as vegetation (black) and lighter pixels as sky (white). Using the EBIImage package (Pau et al. 2010), Canopy openness was then quantified as the proportion of white pixels relative to the total number of pixels in each image. Both metrics were used as proxies for soil protection against rainfall impact and high solar radiation (Akdemir et al. 2016; Kinyili et al. 2024).

Aboveground biomass was estimated using the allometric equation proposed by Chavez et al. (2005) for trees in tropical or moist forest stands. The equation for estimating aboveground biomass (AGB) is described as:

$$AGB = \exp(-2,557 + 0.94 \cdot \ln(\rho D^2 H))$$

Where AGB is the estimated biomass (kg), D is the diameter at breast height (cm), ρ is wood density (g/cm^3), and H is the total height (m) of the evaluated tree. We collected these environmental variables to assess supporting functions (Millennium Ecosystem Assessment, 2005; La Bella et al. 2024). These functions include primary productivity, soil water regulation, soil protection, and nutrient reservoirs (Table 4). In each of the three plots selected to assess the ecosystem function related to soil protection, we also collected all the litter within the 1m^2 subplot.

Table 4 – Variables used as indicators of ecosystem function. P, phosphorus; Zn, Zinc; P-Rem, remaining phosphorus; Cu, copper; Fe, iron.

Ecosystem function	Measured variable
Primary productivity	Aboveground biomass
Soil water regulation	Water holding capacity; Soil electrical conductivity
Soil protection	Litter; Canopy openness
Nutrient reservoir	Soil total concentration of N, P, Zn, P-Rem, Cu and Fe.

For variables that showed a high correlation with other ecosystem function indicators (Pearson's $r > 0.8$), only one variable was selected for further analysis.

3.5 Data analysis

3.5.1 Taxonomic and functional diversity

For each AFS, we calculated both taxonomic and functional diversity. Taxonomic diversity was quantified using species richness, i.e., the total number of species per plot in each AFS. For functional diversity, we constructed a functional space (or morphological space) using probabilistic or n-dimensional hypervolume algorithms. This method enabled us to measure the morphological diversity of species through high-dimensional kernel density estimates. It allowed us to outline the shape and volume of the multidimensional trait distribution while also considering areas in trait space that are unoccupied. As a result, this approach improved the accuracy of our estimates (Blonder et al. 2016).

The traits used in this analysis were grouped as belowground (bl), aboveground (ab), and combined (whole). We first assessed trait correlations and, for those with correlation values above 0.8, we selected only one to avoid multicollinearity. Then, we performed a Principal Component Analysis (PCA) on the standardized (mean = 0, standard deviation = 1) trait data in order to reduce dimensionality. The first three (3) PCA axes, which together explained 95.8%, 93.7% and 72.5% of the belowground, aboveground and total variation, respectively, were then used as input for the hypervolume construction. Thus, the PCA preceded the hypervolume construction.

From the resulting hypervolumes, we extracted three probabilistic functional diversity indices: (i) hypervolume size, representing the functional richness (FRic, the volume of the trait space occupied), (ii) functional dispersion (FDis, the degree of trait dispersion within the space), and (iii) functional evenness (FEve, the uniformity of species distribution in the space). These indices were estimated directly from the hypervolume object using Silverman's bandwidth estimator, which determines the optimal kernel smoothness around species points and a standard quantile threshold. All three indices were evaluated for aboveground, belowground, and integrated trait sets. Hypervolume analyses were performed using the `build.hypervolume` function from the R package 'hypervolume' (Blonder et al. 2016).

To assess our first study focus: how functional traits varied with AFS age, we adopted two complementary approaches. First, at the species level, each species within each AFS was treated as a sampling unit ($n = 33$). We modeled trait values of the sampled species across AFSs age, with species functional traits used as response variables and AFS age as the fixed predictor. Because different species may respond independently, we included species identity as a random factor in the models (Table 5). We examined whether including this component significantly improved the model's explanation power using generalized likelihood ratio tests to compare

nested models. Traits that met the assumptions of normality and homoscedasticity of residuals were analyzed using linear models (LMs), whereas SLA, SRL, and RTD, which did not meet these assumptions, were analyzed using generalized linear models (GLMs) with Gamma distribution.

Table 5. Model selection for each trait. as a response. [in function of the age](#). based on the inclusion of random effects. We compared nested models with and without random effects (species) using likelihood-ratio tests and AIC values. When the inclusion of the random effect did not improve model fit (i.e.. variance close to zero or non-significant likelihood-ratio test). We adopted the simpler model to avoid overfitting.

	Model 1 (simple)		Model 2 (random)		ANOVA
	AIC	logLik	AIC	logLik	p-value
SLA	761.41	-377.70	738.76	-365.38	<0.001
LDMC	-10.278	9.1388	19.060	-6.5302	<0.001
H	43.512	-18.756	44.501	-18.250	0.314
WD	-37.805	21.902	-85.426	46.713	<0.001
SRL	752.58	-373.29	727.16	-359.58	<0.001
RTD	-84.789	45.394	-86.426	47.213	0.056
RN	89.488	-41.744	84.235	-38.118	0.007
RD	-59.158	32.579	-63.525	35.763	0.011

Second, at the community level, the AFS itself was considered the sampling unit ($n = 8$). We calculated the functional identity, quantified by community-weighted means (CWM) of each trait per AFS and tested whether these summary measures varied with system age. In CWM, each species' mean trait value was weighted by its relative abundance in the five AFSs plots. The sum of these weighted values represents the CWM, which reflects the dominant trait values within each community, providing insights into the mechanisms that drive ecosystem services and dynamics (de Bello et al. 2021). The equation below shows how each trait value (X) of a given species (i), multiplied by its relative abundance in the AFS (Fr), determines the CWM value for each AFS.

$$CWM = \sum_{eu=1}^s X_i \cdot Fr_i$$

In both community cases, functional identity (CWM values) and functional diversity indices were treated as response variables, with AFS age as the fixed predictor, and all models were fitted using LMs. This allowed us to compare patterns at both the species and community

levels and to align the structure of trait data with ecosystem function, which was also measured at the AFS level.

3.5.2 Acquisitive-conservative plant ecological strategies

To explore the plant economic spectrum of strategies, we performed a PCA based on species trait values ($n = 33$), which allowed us to reduce trait dimensionality and visualize how species were distributed along the main axes of variation. This ordination was then used to compare the positioning of species between younger and older AFSs. Then, we performed a permutational multivariate analysis of variance (PERMANOVA) to test whether functional differences between AFSs varied significantly according to system age, by comparing recent (1-2 years) and long-term (6-21 years) AFSs. Specifically for this analysis we classified the AFSs in these two categories, which reflects the structure of our monitoring project, in which recently established AFSs are part of a separate ongoing experiment, whereas long-term AFSs represent older, established community-managed systems that serve as reference sites for monitoring. One system of 6 years was considered 'old' due to its low *Musa* sp. presence (which reflect management history), and litterfall patterns, which were closer to other old AFSs than to new systems (Table 1). The analyses were performed separately for aboveground traits (including LA, SLA, LDMC, H, and WD), belowground traits (SRL, RTD, RT, RN and SRA), and for the full set of traits combined. This allowed us to assess whether age-based groupings corresponded to distinct functional strategies, later explored through PCA. PERMANOVA was conducted with 9999 permutations on a Euclidean distance matrix. Since PERMANOVA assumes homogeneity of multivariate dispersions across groups, we additionally applied a permutational analysis of multivariate dispersion (PERMDISP) to ensure that the differences detected by PERMANOVA were not due to unequal within-group dispersion.

3.5.3 Effective Multifunctionality of the AFS ecosystem

First, from the set of soil fertility variables measured in the field, we retained six indicators (P, N, P-Rem, Cu, Fe, Zn) after removing highly correlated variables (Pearson's $r > 0.8$). These were then used to assess ecosystem functioning. Then, the ecosystem functions variables were grouped into four ecosystem functions for each AFS: (i) primary productivity (CWM aboveground biomass), (ii) soil water regulation (water holding capacity, soil electrical conductivity), (iii) soil protection (litter and canopy openness), and (iv) nutrient pool (P, N, Zn, P-Rem, Cu, Fe). Each ecosystem function was calculated using the averaging approach (Byrnes et al. 2014), where ecosystem functions variables (i.e. litter and canopy openness) were

converted into a z-score, and the mean value of these z-scores was used to group as ecosystem function (i.e. soil protection). Then, to define effective multifunctionality we used the approach of Byrnes et al. (2023), which multifunctionality is calculated as the product of effective Hill number of functions and average level of functions. To define the effective number of functions (qN), our 4 ecosystem functions have been standardized to a common scale (i.e. between 0 and 1) and then estimated the level (A) at which the functions are performed (the arithmetic mean of the function values). So we calculated the effective multifunctionality as the product of $qN * A$ (Byrnes et al. 2023; Kinyili et al. 2024). By adopting $q = 1$ (counts functions equally), this approach allowed us to quantify both individual ecosystem functions and the effective multifunctionality in relation to AFS age. To understand these ecosystem functions and multifunctionality in relation to the age of the AFSs, we used the three plots within each AFS to increase the accuracy of the relationship, resulting in a total of 24 sampling units. For all ecosystem functions, this plot-level approach was applied, except for aboveground biomass (AGB), for which we used the community-weighted mean of species biomass at the AFS level ($n = 8$). In addition, we applied LMs with each ecosystem function and the multifunctionality index as dependent variables, and AFS age as the fixed predictor.

In a complementary approach, we used LMs, to understand how functional identity and functional diversity influence ecosystem functioning. For the analyses at the ecosystem function level, we specified each ecosystem function (soil water regulation, soil protection, aboveground biomass, nutrient pool) and multifunctionality as the dependent variable. For aboveground functional identity, the fixed predictors initially included the LA, SLA, LDMC, WD, and H, while for belowground identity they included the SRA, SRL, RTD, RD, and RN. Multicollinearity was checked using variance inflation factors (VIF) according to Zuur et al. (2010), and predictors with inflated values were removed prior to model fitting. The final models therefore retained LDMC, WD, and H as predictors for aboveground functional identity, and SRL, RTD, RD, and RN for belowground functional identity.

All models were validated through visual inspection of residuals, as well as through the Kolmogorov–Smirnov test to verify whether residuals followed a uniform distribution, as expected under the null hypothesis ($p > 0.05$). We also assessed systematic deviations, residual outliers, and dispersion issues (overdispersion or underdispersion). All model validation tools were implemented using the DHARMA package. Additionally, all ecosystem function data were z-standardized before analyses, and Euclidean distance was used to calculate the distance matrix among functional traits. The optimal bandwidth for hypervolume estimation was determined using the `estimate_bandwidth` function (Blonder et al. 2016). Multicollinearity

among predictors in the models was assessed using VIF, with variables showing $VIF > 5$ being removed. All analyses were performed in R software.

4 RESULTS

4.1 Taxonomic diversity

Across the 45 plots of 100 m² evaluated, we recorded a total of 26 plant species, of which 18 were trees, 2 were shrubs; 3 were palms, 1 was a perennial herb, and 1 was a crop. The four most commonly cultivated species were *Theobroma grandiflorum* (Willd. ex Spreng.) K.Schum. (known as Cupuaçu – 71 individuals), *Citrus sp.* (known as Laranja, or orange tree – 47 individuals), *Euterpe oleracea* Mart. (known as Açaí – 26 individuals), and *Coffea sp.* (known as Café, or coffee - 24 individuals).

4.2 Variation in functional trajectories and ecological strategies with AFS age

Among the functional traits evaluated at the species level, only plant height (H) ($R^2 = 0.26$, $p = 0.002$) and LDMC ($R^2 = 0.17$, $p = 0.016$) showed a significant relationship with AFS age, with both increasing as age increased (Fig. 4).

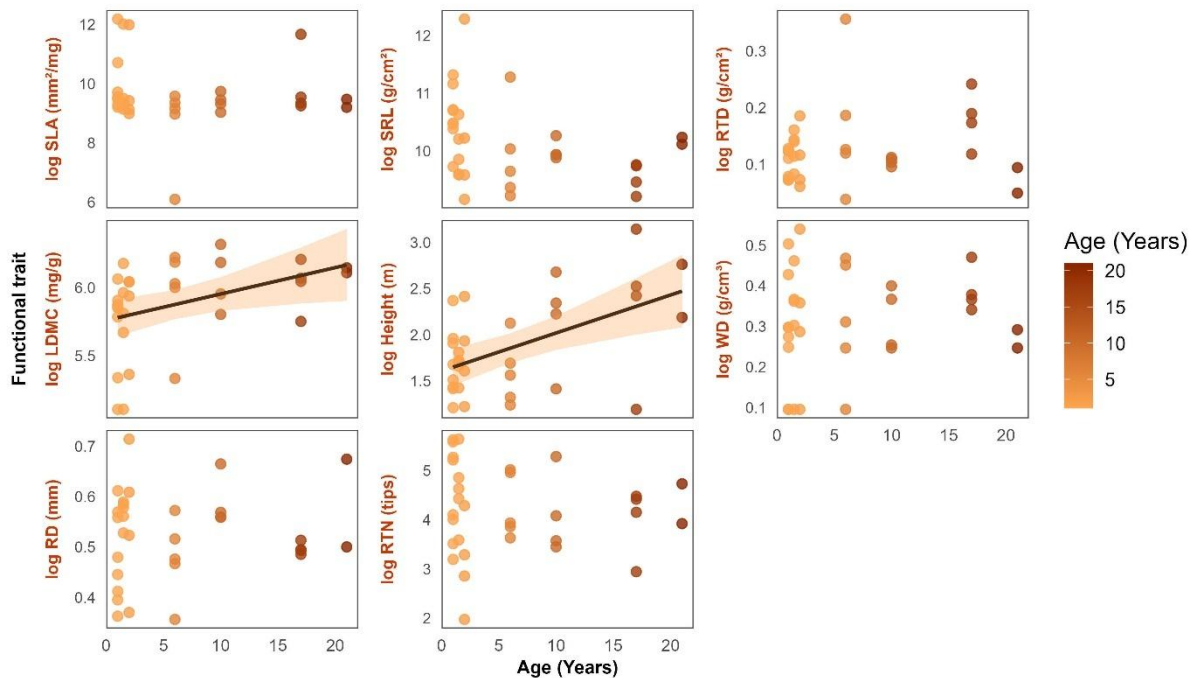


Figure 4. Relationship between functional traits and AFS age at species level. Solid lines indicate statistically significant models ($p < 0.05$). SLA: specific leaf area; LDMC: leaf dry matter content; WD: wood density; H: height; SRL: specific root length; RN: number of root tips; RD: root diameter; RTD: root tissue density.

When assessing the functional identity variation of the AFS (Fig. 5), we also found

significant relationships for both plant height ($R^2 = 0.71$, $p = 0.008$) and LDMC ($R^2 = 0.75$, $p = 0.005$). Other relationships at species or community level were not significant and can be consulted in supplementary material, table S1.

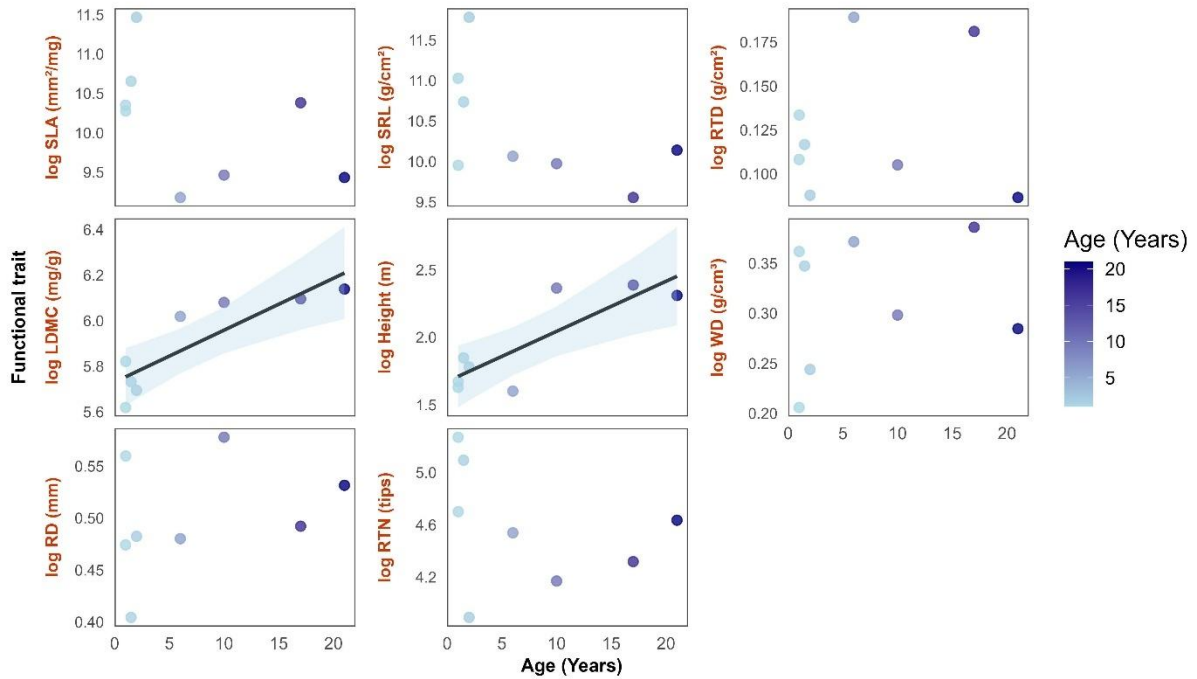


Figure 5. Model showing the relationship between functional identity (represented by CWM) and AFS age. Solid lines indicate statistically significant models ($p < 0.05$). SLA: specific leaf area; LDMC: leaf dry matter content; WD: wood density; H: height; SRL: specific root length; RN: number of root tips; RD: root diameter; RTD: root tissue density.

Our PCA explained 42.08% of the data variation along the first axis and 16.82% along the second axis, totaling 58.89% of the variation. The first PCA axis was strongly influenced by SRA, SRL, LA, LDMC and WD. The second axis was primarily shaped by RTD, RN and SLA, reflecting a paradoxical combination of traits, with acquisitive attributes (SLA and RN) occurring alongside high RTD (a conservative trait) (Fig. 6). Overall, the ordination reveals a clear gradient from acquisitive to conservative strategies, with the negative side of the plot (both upper and lower quadrants) associated with acquisitive traits, and the positive side (upper and lower quadrants) associated with conservative traits.

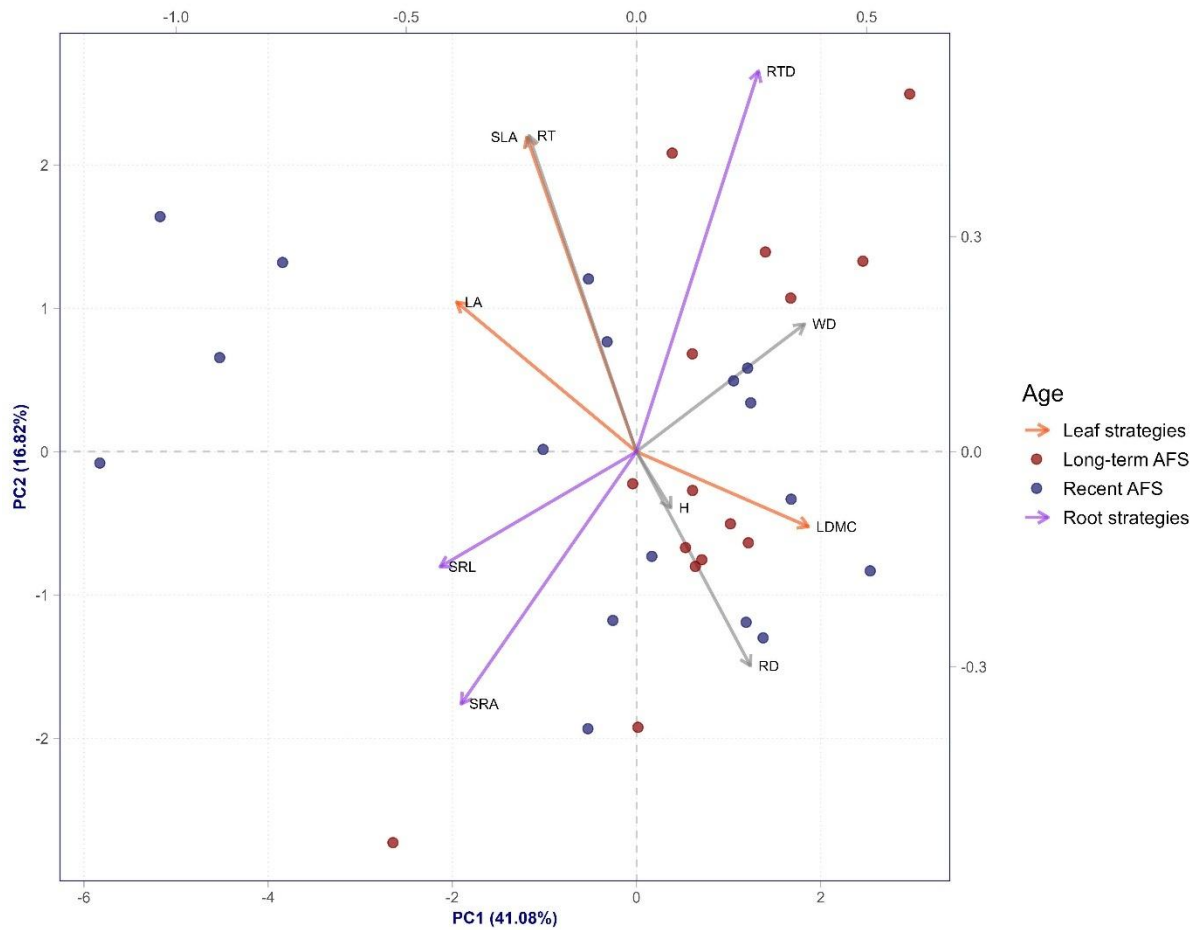


Figure 6. PC1 has an eigenvalue of 3.36 and represents the trade-off in belowground resource investment (hereafter ‘Root strategies’), indicated by the purple arrows of SRL and SRA and leaf ecological strategies (hereafter ‘leaf strategies’), indicated by the orange arrow of LDMC. PC2 has an eigenvalue of 1.34 and reflects the leaf strategies (orange arrow: SLA), together with root strategies (purple arrow: RTD). LA: Leaf Area; SLA: Specific Leaf Area; LDMC: Leaf Dry Matter Content; H: Height; RTD: Root Tissue Density; RD: Root Diameter; RT: Number of Root Tips; SRL: Specific Root Length; WD: Wood Density.

Species in long-term AFSs tended to be associated with conservative strategies (high RD, RTD and LDMC), whereas while some species in recent AFSs were associated to acquisitive strategies (high SLA, RT, SRL and SRA), showed no clear separation, likely due to the strong influence of *Musa sp.*, that presented high LA and SLA values. To address this, we re-ran the PCA excluding *Musa* (Supplementary material – Fig. S1), which orthogonal root and leaf axis confirmed that recent AFSs were associated with acquisitive traits (higher SRL, SRA and SLA), while long-term AFSs exhibited more conservative attributes (higher LDMC, RTD and plant height).

The PERMANOVA confirmed a significant ecological separation between long-term (6-20 years) and recent (1-2 years) AFSs for aboveground functional traits (Pseudo F: 3.638, p

= 0.0152) and considering all the set of traits (Pseudo F: 2.528, $p = 0.030$). The PERMDISP indicated that the variation was not driven by heterogeneous sampling between the two groups for aboveground traits ($F: 0.074$; $p = 0.787$) and considering all the set of traits ($F: 0.276$; $p = 0.602$), thus increasing the reliability of our analysis. The permutational analysis did not detect significant differences between root trait groups (Pseudo F: 1.571, $p = 0.179$; SM - Table S2).

4.3 Functional dynamics of the ecosystem

In our analysis of the variation in ecosystem functions, we found that the aboveground biomass ($p = 0.004$, $R^2 = 0.31$), nutrient pool function ($p = 0.03$, $R^2 = 0.46$) and soil protection ($p < 0.001$, $R^2 = 0.57$) were significantly affected by the age of the AFS. Furthermore, we found that the number of effective functions ($p = 0.03$, $R^2 = 0.2$) and the effective multifunctionality ($p < 0.001$, $R^2 = 0.57$) increases with AFSs age (Fig. 7, SM - Table S3).

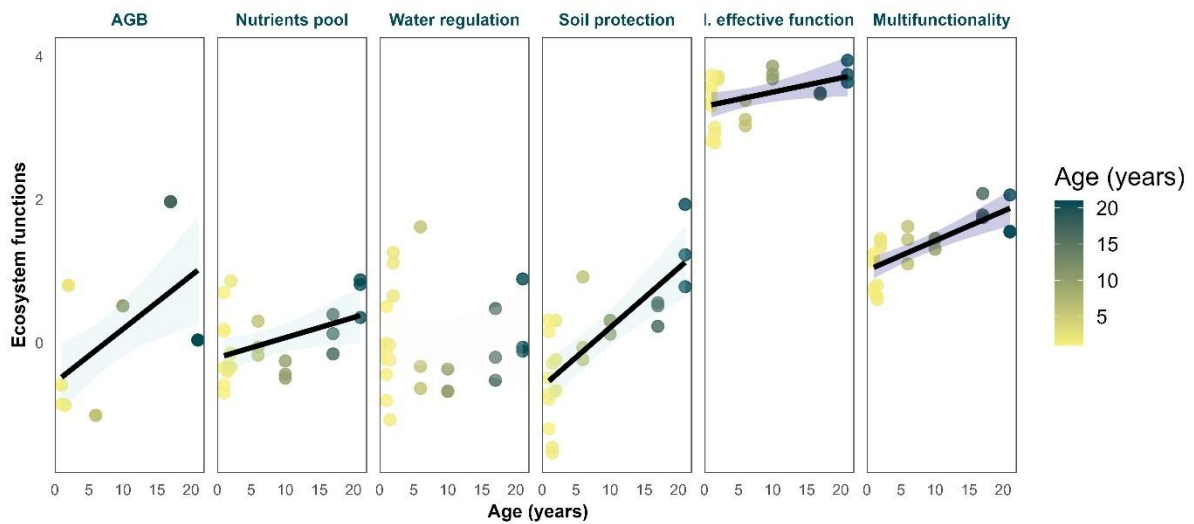


Figure 7. Behavior of ecosystem functions with AFS maturation. Solid lines indicate statistically significant models ($p < 0.05$). AGB: aboveground biomass.

Among the variables used to understand ecosystem functioning, we observed significant relationships between AFS age and factors such as canopy openness, Cu, available P, leaf litter, and Zn (Fig. 8, SM - Table S4).

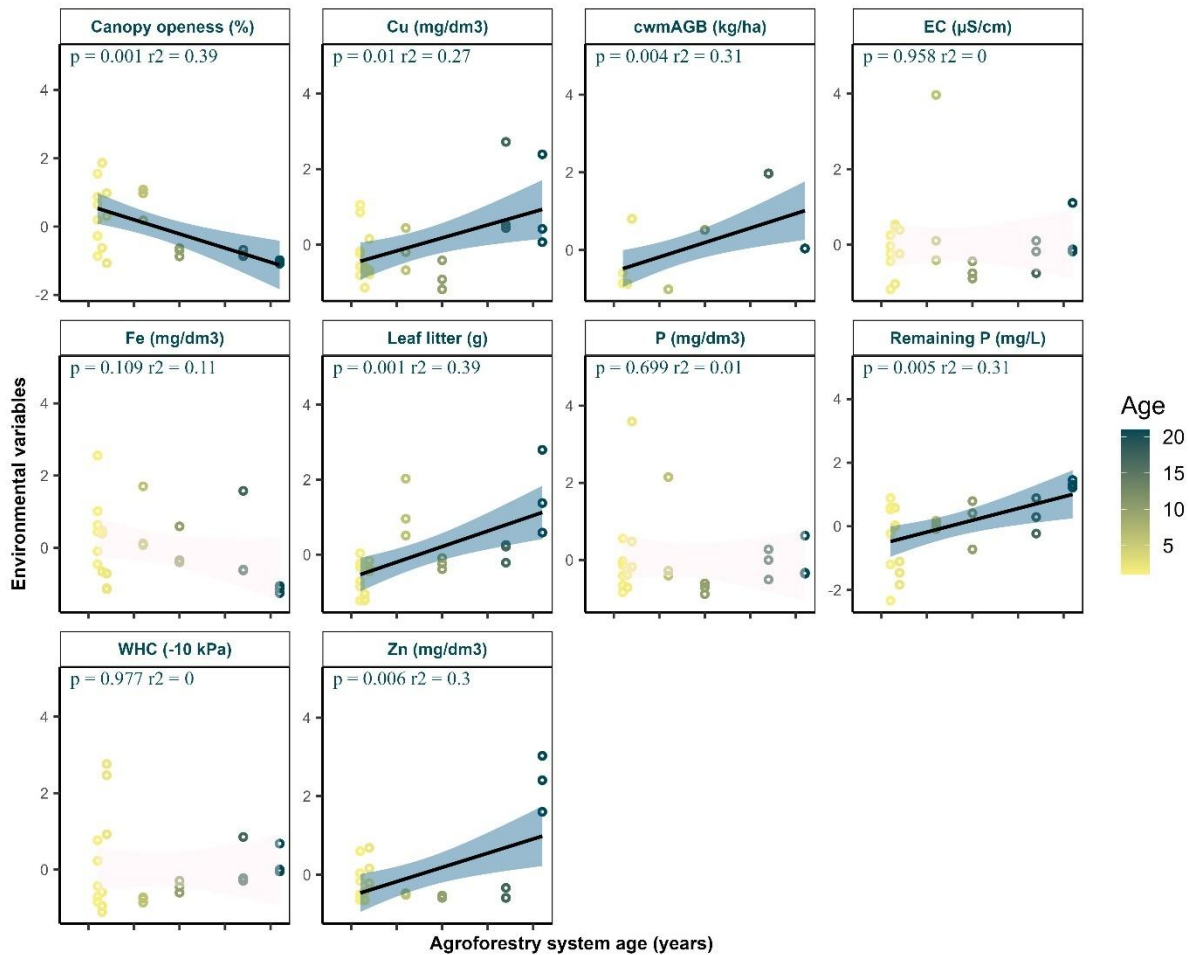


Figure 8. Relationship between the age of Agroforestry Systems and individual indicators of system functioning. Solid lines indicate model significance. Cu: copper; EC: electric conductivity; Fe: iron; P: phosphorus; WHC: water holding capacity; Zn: zinc.

Despite no direct interaction with the age gradient, we also found that the functional identity for LDMC was associated with AFS ecosystem functioning. Specifically, LDMC was associated with greater soil protection. This pattern was particularly evident in long-term AFSs, where higher LDMC values coincided with higher values of soil protection function. However, the relationship between LDMC (independent variable) and soil protection (dependent variable) did not appear to be linear, which may reflect either limited sample size or non-linear ecological responses. To address this, we classified the AFSs into “new” and “old” (as in the PCA analysis) and tested for differences in their multivariate functional composition using a PERMANOVA (pseudo-F: 63.581; $p = 0.02$; Fig. 9). Other direct relationships between functional identity and ecosystem functions were not significant.

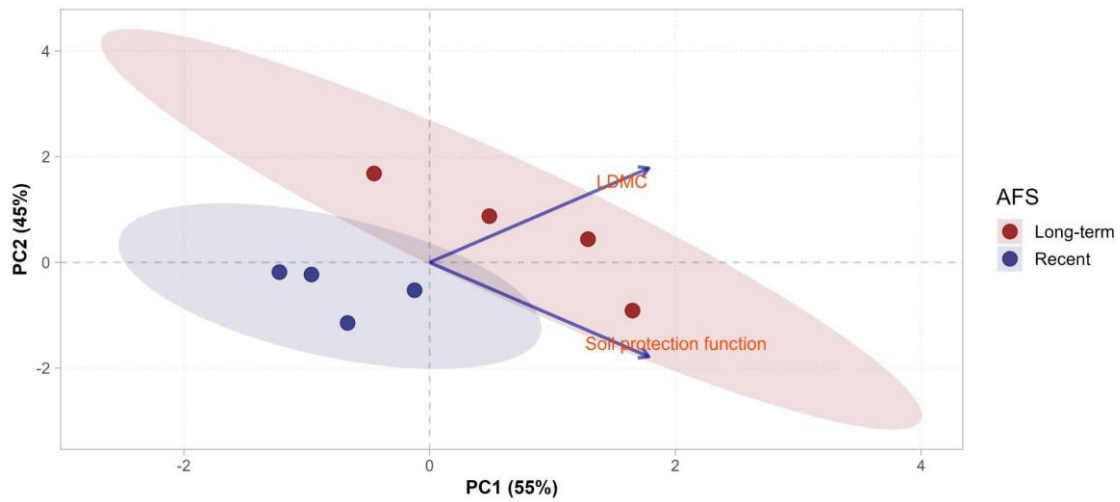


Figure 9. Multivariate comparison of CWM LDMC and soil protection function between recent (blue) and long-term (red) AFSs. PERMANOVA's pseudo-F = 63.581 and p value = 0.02.

4.4 Functional diversity in Agroforestry Systems

Only the aboveground functional dispersion showed a negative relationship with AFS age (Fig. 10). None of the other functional indices were influenced by the agroforestry systems' age (SM - Table S5). Also, the functional diversity indices did not show a direct relationship with ecosystem functions (SM – Table S6).

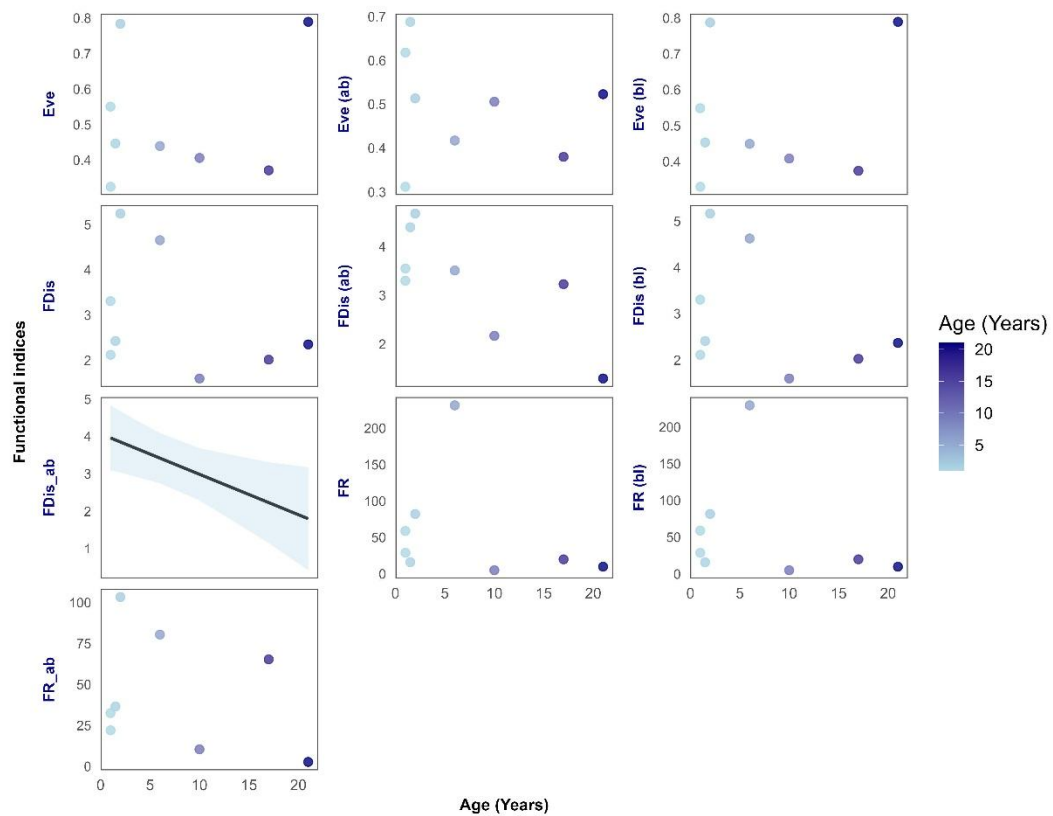


Figure 10. Relationship between functional diversity and age in agroforestry systems. Solid lines represent statistically significant effects ($p < 0.05$). FR refers to functional richness, or hypervolume; FEve represents functional evenness; and FDis denotes functional dispersion. Additionally, indices labeled with “ab” correspond to traits measured aboveground, whereas those marked “bl” refer to belowground traits.

5. DISCUSSION

Our study reveals that the human-driven functional composition of tree communities in AFS in eastern Amazonia shifts consistently with system age, reflecting a transition from acquisitive to more conservative ecological strategies. As AFSs age increases, we also observe improvements in ecosystem functions related to soil fertility, soil stability, aboveground biomass storage and effective multifunctionality, which may reduce dependence on external inputs and enhance climate resilience. Importantly, the dominance of some functional traits, such as LDMC, plays a key role in the provision of ecosystem services stability, thus playing a central role in the ecosystem functioning. Meanwhile aboveground dispersion decreases with system age, which reflects the filtering of species driven by human management.

5.1 The role of the shift in functional traits and ecological strategies are driven by the Agroforestry Systems age and management

As we hypothesize, ecological strategies vary according to the age of AFSs. In AFSs, age reflects not only the time elapsed since establishment, but also the historical management decisions that strongly shape their functional trajectories. The variation in height and LDMC at both community and species level with AFS age reinforces a functional reorganization of the community along the functional trajectory of the species. This pattern aligns with the leaf and root economics spectra (Wright et al. 2004; Reich et al. 2014; Bergmann et al. 2020), which propose trade-offs between acquisition and conservation strategies across plant organs. We highlight that temporal functional shift observed in our AFS age sequence reflects a successional trajectory that reflects processes described in natural forest recovery (Poorter et al. 2016; Vicente-Silva et al. 2016). Beyond these processes similar to successional recovery which impacts the shift in functional trajectories, in our AFSs these changes are anticipated by human management, highlighting the role of AFS designers in making management choices across the different stages of the system. LDMC and tree height were particularly responsive to AFS age. Low LDMC in recent AFSs suggests rapid turnover and short leaf lifespan, optimizing photosynthesis and productivity under high light availability (Wright et al. 2004). In contrast, in long-term AFSs, higher LDMC reflects leaf structural longevity, reduced leaf damage, and

lower leaf turnover costs (Reich et al. 2014). This strategy is associated with shadier environments and more stable microclimates where light limitation imposes high costs for producing new leaves, making it advantageous to maintain durable leaf structures (Carreño-Rocabado et al. 2012), but also reflects the role of the smallholders as decision makers in ecological process of the AFSs. Around the fourth or fifth year, the smallholders in the RESEX Chico Mendes often remove the *Musa* sp. species, which have been used to provide shade for other plant species before. With these changes, they anticipate ecological strategies that would otherwise emerge only after many years in natural (Navas et al. 2009; Raavel et al. 2012) and managed areas (Garnier et al. 2018).

The increase in tree height with AFS age supports the balanced-growth hypothesis (Garnier 1991), which proposes that resource allocation shifts toward the organ where resources are most limiting for growth, in this case to vertical structural investment. This vertical functional reorganization may enhance competitiveness as agroforestry systems mature, aligning with patterns observed in later successional trajectories (Shipley and Meziane, 2002; Silva et al. 2019). This reveals that height is a key functional trait in AFSs dynamics (Thomas et al. 2021; Lee et al. 2024). Interestingly, not all traits responded to system age. This stability could reflect either consistent functional strategies over ontogeny (Garbowski et al. 2021) or improved edaphic conditions by AFS management over time, such as soil fertility and moisture retention. According to Lavorel and Garnier (2002), some traits that consistently vary across environmental gradients are identified as "response traits". Given their connection to resource-use strategies, future research focused on these traits for the species studied here may provide insights into tree establishment, dynamics, and survival, regardless of AFS age.

While individual traits such as tree height and LDMC responded differently to AFS age, a broader multivariate perspective revealed two main axes of functional variation that encompass integrated whole-plant strategies. These two primary axes of functional variation represent distinct but complementary functional dimensions. Individuals in more recent AFS exhibited predominantly acquisitive strategies, characterized by low investment and rapid return, whereas long-term AFSs, driven by human actions, showed a transition to more conservative strategies, associated with high investment and slow return (Wright et al. 2004). Two groups of ecological strategies can be highlighted in these AFSs: the leaf characteristics, such as LDMC, SLA, tree height, which define a continuum of resource acquisition and conservation strategies. This pattern is well described in the literature as the leaf economics spectrum (Wright et al. 2004; Chave et al. 2009) and has been expanded in later studies to include broader structural and reproductive aspects (Reich 2014). The second functional group

encompasses root traits, with a pattern of variation revealing a pattern of variation analogous to the leaf economics spectrum (Wright et al. 2004) and aligned with the root economics spectrum and broader economic frameworks proposed in later studies (Reich, 2014; Bergmann et al., 2020). This integrative view is particularly relevant in managed ecosystems like AFSs, where both above and belowground components mediate processes such as productivity, nutrient cycling and resilience (Freschet et al. 2021). By capturing these multiple dimensions, our approach provides a more mechanistic understanding of functional reorganization in response to management and time.

5.2 The age of agroforestry systems leads to increased multifunctionality and the provision of ecosystem services

AFSs have long been recognized for their multifunctional potential, but our results reveal that system age acts as a key integrative driver of ecosystem functioning. Contrary to the many studies that focus on understanding the role of biodiversity in ecosystem multifunctionality (Isbell et al. 2011; Byrnes et al. 2023; La Bella et al. 2024), we observed relatively constant richness across our systems (around 4-5 species), while multifunctionality increased with age. These points reinforce the importance of temporal ecological processes, such as canopy development, litter accumulation, and trait filtering in structuring functional outcomes, reflecting the need for adequate species pool, a dimension that remains underexplored in studies on the subject. While studies such as Byrnes et al. (2014) and van der Plas et al. (2016) highlight the role of species richness in sustaining ecosystem services, our findings point to a complementary mechanism: in line with the temporal insurance effect proposed by Yachi and Loreau (1999), AFSs become more stable and resilient not only through biodiversity itself, but also through human-shaped by structure and the time-driven accumulation of ecological interactions and feedback. This contributes to building more stable, resilient, and functionally diverse systems. Our findings are consistent with management approaches that monitor long-term land recovery (Yu & Wang, 2018; Wang et al. 2020). However, none of these studies have explicitly addressed the role of effective multifunctionality patterns. This aspect remains underexplored in management systems, specifically in agroforestry literature, and thus our results contribute to the theoretical understanding of multifunctionality beyond biodiversity metrics.

Furthermore, not only age drives changes in the effective multifunctionality in AFSs. While other agroecological studies did find associations between functional identity and some

ecosystem functions, such as aboveground biomass (Noulèkoun et al. 2023), functional identity here also played a relevant role in modulating ecosystem services across our AFSs of different ages. We found that LDMC, a trait linked to conservative ecological strategies, was positively associated with soil protection function in long-term AFSs. LDMC is often associated with higher structural investment (De Bello et al. 2010), and in our study, this likely translates into greater leaf persistence and protective effect of standing foliage against erosion, desiccation, and pathogen spread (Meylan et al. 2017; Avelino et al. 2023). This trait, while traditionally considered response traits (Larcher 2003; De Bello et al. 2021), acted as effect traits in this context, contributing to ecosystem protection by increasing resistance both aboveground - through thicker, more persistent leaves in the canopy - and at the soil surface, where higher LDMC may also enhance the protective role of leaf litter.

Long-term AFSs showed clear signs of improved soil fertility along the AFS-age, with enhanced nutrient pool function, as well as higher levels of zinc (Zn), copper (Cu), and remaining phosphorus (P-rem). These micronutrients are essential to plant physiology and are typically limited in tropical soils with acidic pH (White & Brown, 2010), such as those in the Amazon region. According to smallholders, chemical fertilization was applied in long-term AFSs only once, at the beginning, in two systems, while the other two never received any fertilization at any stage. While we cannot rule out a residual fertilization effect, we assume that the observed increases likely result from enhanced litter production, microclimatic stabilization, and biological activity over time, supporting more effective nutrient cycling and retention (Souza et al. 2025). This aligns with findings from other succession studies, where nutrient pools recover as vegetation matures and soil structure improves (Nesper et al. 2019). From an applied perspective, our results suggest that, in the RESEX CM context, AFSs aging enhances internal nutrient recycling, which may reduce the dependence on external inputs, such as phosphate fertilization. This reduction not only contributes to more sustainable management practices but may also lessen production costs for smallholders.

In our agroforestry systems, above-ground biomass increases over time, a pattern consistent with Grime's mass ratio hypothesis (1998), which posits that the species with the highest abundance disproportionately affect ecosystem functioning. In early-stage AFSs, farmers plant high-density, fast-growing species such as *Musa* sp., which have low biomass accumulation, but provide both shading for companion species and food for subsistence. Although these early successional species dominate community biomass in the short term, they are typically removed after approximately four years, allowing high-biomass species, such as *Hevea brasiliensis*, to consolidate. This shift in dominance results in an increase in biomass

over time. Thus, the temporal management of fast-growing species facilitates biomass accumulation, contributing directly to carbon storage and supporting global climate mitigation goals (United Nations, 2015) and highlights the potential role of human-driven filtering of species in regulating the effect of carbon storage in human-modified landscapes (Noulèkoun et al. 2023).

5.3 From Functional Dispersion to Convergence: Implications for AFS resilience

Our study provides novel insights into the role of functional diversity in AFS across different ages in western Amazon. While we found no significant effects of system age on many facets of functional diversity, we observed a decrease in functional dispersion with increasing AFS age. This suggests that older systems are composed of species with more similar ecological strategies. This pattern may reflect human-driven filtering of species in agroforestry systems, where farmers tend to favor or maintain species with desirable traits, thereby constraining the functional trait space over time (Noulèkoun et al. 2023). This challenges the assumption that age alone is a reliable proxy for vegetation functional development in managed tropical systems. Additionally, functional redundancy among species may contribute to the early saturation of trait space (Fukami et al. 2005). If the species introduced or favored in both recent or long-term AFSs share similar ecological strategies, particularly traits associated with productivity and resource-use efficiency, the functional trait space may be saturated early in system development. This would limit detectable changes over time, despite structural differences or biomass accumulation.

Furthermore, trait expression may be driven more by local abiotic variation, such as microclimate, soil characteristics, or organic matter input (Vicente-Silva et al. 2016), than by system-level characteristics like age. Particularly, environmental heterogeneity and high plasticity may mask temporal trends in the traits (Schneider & Lynch, 2020). This calls for greater attention to fine-scale environmental filters and the potential decoupling between above- and below-ground functional dynamics. Indeed, increasing system age alone may not sufficiently enhance functional diversity or key variation in functional complementarity. For land managers and policymakers, this shows the importance of intentional design and maintenance strategies focused on trait complementarity and environmental heterogeneity. Furthermore, promoting diverse functional types through planting schemes or soil enrichment could be more effective than relying on time to drive ecological assembly.

6. CONCLUSION

In our study systems, management interventions are particularly intense in the first years, when farmers remove short-lived crops and this process establishes long-lived species. After this stage, management decreases substantially, and ecological filters become the main drivers of community assembly. This reinforces the idea that management can act as an ecological designer, accelerating functional shifts that favor ecosystem services and resilience. We evaluated the functional diversity, ecological strategies and multifunctionality of agroforestry systems, considering the influence of system age. Older systems exhibited more conservative strategies and higher levels of ecosystem multifunctionality, including enhanced soil fertility, carbon storage and protection. Despite reduced trait dispersion, indicating ecological convergence, these systems maintained high functional performance through trait combinations that favor resource conservation and resilience. LDMC was consistently linked to stability, highlighting its key role in AFS ecosystem functioning. Moreover, decreases in aboveground dispersion with system age suggests that older AFSs are composed of species with similar ecological strategies, which may limit opportunities for complementary trait effects. Our findings reinforce the potential of mature agroforestry systems to optimize multiple ecosystem services, making them a powerful nature-based solution for sustainable land management and climate adaptation in tropical landscapes.

7. CONSIDERAÇÕES FINAIS

Promover espécies funcionalmente diversas, incluindo aquelas com estratégias conservadoras em sistemas de longo prazo, pode aumentar a estabilidade do ecossistema, reduzir os custos de rotatividade e apoiar o fornecimento de longo prazo de serviços ecossistêmicos, como a manutenção da fertilidade do solo e a regulação do microclima. Isso tem implicações diretas para programas que promovem a agricultura sustentável em regiões tropicais, especialmente em áreas degradadas ou anteriormente desmatadas. Além disso, as atuais políticas e incentivos agroflorestais, que frequentemente se concentram na composição de espécies ou na biomassa acima do solo, sem considerar características funcionais ao nível das raízes, podem - integrando espécies com estratégias radiculares contrastantes - aumentar a complementaridade abaixo do solo, o que pode promover a resiliência em períodos de escassez hídrica. Esse planejamento baseado em características poderia apoiar o refinamento de recomendações técnicas para a implementação de SAFs em programas regionais de restauração e segurança alimentar.

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SUPPLEMENTARY INFORMATION

Age-driven shifts in functional composition and diversity shape ecosystem service provision in agroforestry systems

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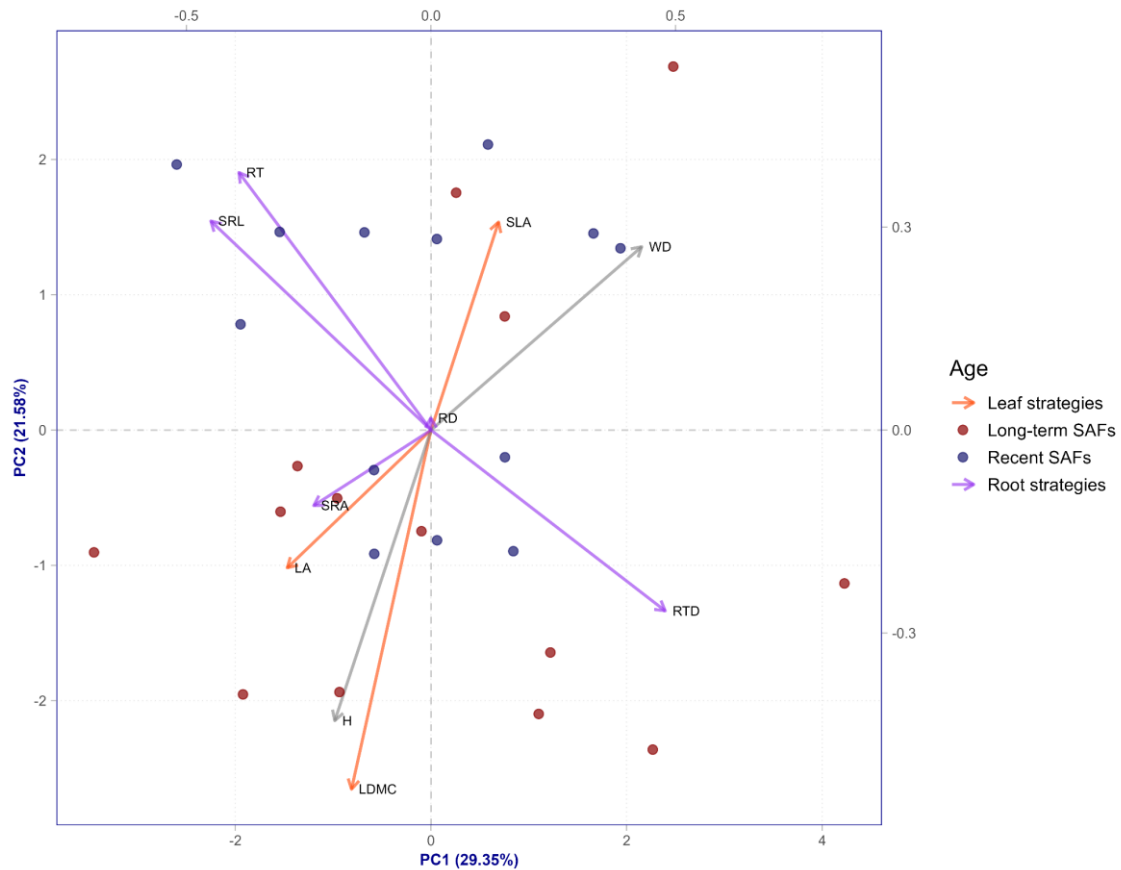


Figure S1. PC1 has an eigenvalue of 2.93 and represents the trade-off in belowground resource investment (hereafter ‘Root strategies’), as indicated by the purple arrows (SRL, RT, and RTD), while PC2 has an eigenvalue of 1.91 and reflects leaf ecological strategies (hereafter ‘leaf strategies’), indicated by the orange arrows (SLA, LDMC). LA: Leaf Area; SLA: Specific Leaf Area; LDMC: Leaf Dry Matter Content; H: Height; RTD: Root Tissue Density; RD: Root Diameter; RT: Number of Root Tips; SRL: Specific Root Length; WD: Wood Density.

Table S1. Summaries of linear model and generalized linear model between each functional trait and the gradient age of the agroforestry system in the Western Amazonia. Chico Mendes Extractive Reserve. Acre. Brazil. If a random factor was used can be assessed in table S5). KS test. residual uniformity test; Disp. residual dispersion test; AIC. Akaike information criterion. Significant effects are highlighted in bold with *.

SPECIES LEVEL				
Linear model				
Trait	R ²	p-value	KS test	Disp. test
LDMC	0.191	0.010*	0.437	0.96

H	0.039	0.002*	0.988	0.96	
WD	0.017	0.466	0.921	0.96	
RTN	-	0.276	0.944	0.88	
RD	0.020	0.426	0.976	0.96	
Generalized linear model					
Trait	AIC	Estimate	p-value	KS test	Disp. test
SLA	738.76	0.013	0.589	0.094	0.14
SRL	727.2	-0.017	0.223	0.818	0.79
RTD	-86.4	0.006	0.672	0.303	0.96
COMMUNITY LEVEL					
Linear model					
Trait	R ²	p-value		KS test	Disp. test
SLA	0.233	0.225		0.928	0.92
LDMC	0.790	0.003*		0.637	0.92
WD	0.040	0.633		0.529	0.92
H	0.712	0.008*		0.990	0.92
SRL	0.329	0.137		0.848	0.92
RTD	0.007	0.835		0.768	0.92
RTN	0.148	0.346		0.792	0.92
RD	0.109	0.422		0.711	0.92

Table S2 - Summaries of PERMANOVA and PERMDISP model between each functional group traits and the Age of the agroforestry system.

PERMANOVA of Aboveground traits				
<i>Indices</i>	<i>Sum Sq</i>	<i>R²</i>	<i>F value</i>	<i>p - value</i>
Groups	13.413	0.1081	3.638	0.016*
Residuals	110.587	0.8918	-	-
PERMDISP of Aboveground traits				
Groups	0.09	-	0.074	0.787
Residuals	36.48	-	-	-
PERMANOVA of Belowground traits				
Groups	7.717	0.0497	1.571	0.175
Residuals	147.283	0.9502	-	-
PERMDISP of Belowground traits				
Groups	1.623	-	0.864	0.359
Residuals	56.309	-	-	-
PERMANOVA of Plant traits				
Groups	19.275	0.0777	2.528	0.030*
Residuals	228.725	0.9222	-	-
PERMDISP of Plant traits				
Groups	0.555	-	0.276	0.602
Residuals	60.167	-	-	-

Table S3. Summaries of ecosystem function across the agroforestry system age gradient availed by linear models. KS test, residual uniformity test; Disp, residual dispersion test; Significant effects are highlighted in bold.

Ecosystem function	R ²	p-value	KS test	Disp. test
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Aboveground biomass	0.31	0.004	0.3168	0.92
Water regulation	0.01	0.615	0.1890	0.92
Soil protection	0.57	0.001	0.1578	0.92
Nutrientes pool	0.46	0.001	0.1649	0.92
N. effective functions	0.19	0.030	0.8497	0.92
Multifunctionality	0.57	< 0.001	0.4994	0.92

Table S4. Summaries of variables of ecosystem across the age gradient in agroforestry systems. WHC, water holding content; EC, electric conductivity; P, phosphorus; Zn, Zinc; Remaining P, remaining phosphorus; Cu, copper; Fe, iron. KS test, residual uniformity test; Disp, residual dispersion test; AIC, Akaike information criterion. Significant effects are highlighted in bold.

Ecosystem variable	R ²	p-value	KS test	Disp. test
AGB	0.31	0.004	0.3168	0.92
WHC	0	0.977	0.4469	0.92
EC	0	0.958	0.1148	0.92
Leaf litter	0.39	0.001	0.6113	0.92
Canopy opening	0.39	0.001	0.9225	0.92
P	0.01	0.699	0.0889	0.92
Zn	0.3	0.006	0.8145	0.92
Remaining P	0.31	0.005	0.2998	0.92
Cu	0.27	0.01	0.307	0.92
Fe	0.11	0.109	0.5228	0.92

Table S5 - Summaries of linear model between functional diversity indices and the gradient age of the agroforestry system in the Western Amazonia, Chico Mendes Extractive Reserve, Acre, Brazil. Significant effects are highlighted in bold. FR, Functional richness (functional hypervolume); FDis, Functional dispersion; FEve, Functional evenness; bl - belowground morphological diversity; ab - aboveground morphological diversity

Indice	AIC	Estimate	Std. error	t-value	p-value	R ²	Shapiro
FR	28.31	-0.071	0.054	-1.324	0.233	0.23	0.970
FDis	30.73	-0.067	0.063	-1.076	0.323	0.16	0.118
FEve	-0.13	0.004	0.009	0.533	0.612	0.04	0.074
FR (ab)	84.07	-1.296	1.770	-0.732	0.491	0.08	0.584
FDis (ab)	21.98	-0.108	0.036	-2.962	0.025*	0.59	0.560
FEve (ab)	-6.23	-0.003	0.006	-0.532	0.614	0.04	0.921
FR (bl)	28.28	-0.071	0.054	-1.323	0.234	0.22	0.966
FDis (bl)	7.36	-0.015	0.014	-1.08	0.320	0.16	0.320
FEve (bl)	-0.244	0.004	0.009	0.523	0.619	0.04	0.072

Table S6. Summaries of the impact of functional diversity in the provision of ecosystem functions. Significant effects are highlighted in bold. FR: Functional Richness. FDis: Functional dispersion. FEve: Functional Evenness. FEve total (Aboveground + Belowground) was not used due to high collinearity within the model with other two predictors.

		<i>Aboveground Dis</i>		<i>Aboveground FEve</i>	
Function		Estimate	p-value	Estimate	p-value
Aboveground biomass		-0.517	0.142	-3.252	0.276
Water regulation		0.033	0.879	-0.310	0.873
Soil protection		-0.013	0.963	-1.677	0.544
Nutrients pool		-0.021	0.902	0.645	0.687
Multifunctionality		-0.109	0.502	-0.515	0.719
<i>Model's R²: 0.52; 5 DF</i>					
		<i>Belowground FR</i>		<i>Belowground FEve</i>	
Function		Estimate	p-value	Estimate	p-value
Aboveground biomass		< 0.001	0.918	2.745	0.287
Water regulation		< 0.001	0.860	-1.217	0.328
Soil protection		0.006	0.077	0.082	0.951
Nutrients pool		0.002	0.277	-0.808	0.389
Multifunctionality		0.001	0.472	0.286	0.768
<i>Model's R²: 0.22; 5 DF</i>					