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ABSTRACT

Agroforestry systems (AFS) can enhance biodiversity and ecological recovery in degraded agricultural landscapes. We evaluated spontaneous vegetation and fine- and coarse-root biomass in five young AFS modules established on degraded pasture in southeastern Pará, Brazil, using adjacent secondary forest as an ecological reference. One year after AFS establishment, spontaneous plants ≤1.5 m tall were surveyed and root biomass sampled at 0–10 cm depth. Species richness and individual density differed among AFS modules, whereas root biomass did not. The forest had substantially greater fine- and coarse-root biomass (284.0 and 58.4 g m^{-2}, respectively) than AFS modules (56.3–116.0 and 1.5–7.2 g m^{-2}). Across the forest–AFS gradient, root biomass was negatively associated with spontaneous-vegetation richness and abundance, while life-form composition was strongly associated with root biomass (RDA, adjusted $R^2 = 0.66$; $p < 0.001$). These relationships were not detected in AFS-only analyses, indicating that above–belowground associations were more clearly expressed across the broader forest–AFS gradient than among young AFS alone. Because root taxonomic identity was not determined, these patterns represent ecosystem-level covariance rather than direct contributions of particular spontaneous life forms. Spontaneous vegetation differentiated rapidly during early AFS development, whereas belowground biomass remained similar among young AFS configurations. For smallholder agroforestry, spontaneous vegetation may provide an early indicator of ecological reorganization, while belowground differentiation may require longer-term monitoring. These findings support recognizing spontaneous vegetation as a component of ecological recovery rather than solely as vegetation to be suppressed.

KEYWORDS: Ecological restoration, fine roots, forest reference, natural regeneration, plant diversity, vegetation life forms

INTRODUCTION

Family or subsistence farming is one of the predominant forms of land use across the Amazon region (Costa et al. 2024). Traditionally, land preparation relies on the slash-and-burn system, in which primary or secondary forests are cut and burned to establish crops, initiating short cultivation cycles that persist only until soil fertility declines (Abrell et al. 2024; Bezerra et al. 2024). Historically, this system maintained a certain ecological balance through alternating phases of cultivation and fallow, during which abandoned areas regenerated naturally via secondary succession, partially restoring fertility and enabling new agricultural cycles (Siminski and Fantini 2007; Steenbock et al. 2021). However, increasing demographic pressure and the reduction of available land for new clearings have progressively shortened fallow periods, intensifying land degradation and undermining the sustainability of smallholder agriculture (de Holanda Leite 2022; Kuchler et al. 2022; Vieira and Proctor 2007). As a result, large areas of the southeastern Amazon now consist of degraded pastures and secondary forests with limited recovery potential, marked by soil erosion, nutrient loss, and carbon depletion (Souza Braz et al. 2013). In response to this scenario, agroforestry systems (AFS) have emerged as a sustainable alternative to the cycle of degradation, integrating trees and other perennial species with crops and/or livestock to restore ecological functions while maintaining productivity (Keprate et al. 2024). For smallholder landscapes, their relevance lies not only in diversifying production but also in promoting vegetation recovery and rebuilding ecological processes on previously degraded land. These systems enhance biodiversity, improve soil fertility, and can contribute to carbon storage (Jose and Bardhan 2012; Isaac and Borden 2020). However, considerably less is known about how the spontaneous vegetation developing within young AFS is associated with belowground biomass during early AFS development, particularly whether its richness, density, and life-form composition are related to fine- and coarse-root biomass.

By integrating trees and other perennial woody species with agricultural crops and/or livestock, AFS promote greater structural and biological diversity than conventional monocultures (Nair 1993; Dmuchowski et al. 2024). This diversification supports high species richness, improves habitat heterogeneity, and enhances ecological functions such as pollination, seed dispersal, soil fertility, and soil protection (Barbosa et al. 2026; Nair et al. 2021). Moreover, the coexistence of multiple vegetation strata, comprising upper, middle, and lower canopy layers, enables AFS to store considerable amounts of carbon in plant biomass and soil organic matter (Jose and Bardhan 2012; Rocha et al. 2014; Ramos et al. 2017; Santos et al. 2019). However, the ecological performance of AFS is determined not only by the species intentionally planted by farmers. As these systems develop, naturally recruiting herbs, shrubs, trees, palms,

and lianas form an additional vegetation component whose structure may respond to canopy development, management, and local environmental conditions. This spontaneous vegetation can therefore provide information about early ecosystem reassembly and may also interact with belowground biomass development. Despite growing evidence of the ecological benefits of AFS, most assessments have focused on aboveground components, with comparatively less attention to spontaneous flora and belowground biomass, particularly fine roots, which play important roles in nutrient acquisition, organic-matter inputs, and belowground carbon cycling.

Spontaneous vegetation is a particularly relevant but often overlooked component of young agroforestry systems. AFS can support a rich and functionally diverse spontaneous flora emerging between crop rows and recruiting from propagule sources including the soil seed bank and surrounding vegetation, including tree and shrub species important for ecological succession and natural regeneration (Lacerda et al. 2013, 2016). Because this vegetation can respond rapidly to changes in resource availability, disturbance, management, and the developing structure of the planted community, its composition provides an important indicator of ecological reorganization during the early stages of AFS establishment. This vegetation layer is not merely a by-product of management practices but a dynamic component that contributes to ecosystem recovery by protecting the soil surface, reducing erosion, and improving microclimatic and hydric conditions. Through continuous litter input and root turnover, spontaneous species can also contribute to nutrient cycling and organic-matter accumulation in degraded areas (Lacerda et al. 2016). Furthermore, Ramos et al. (2015) demonstrated that the arrangement and composition of planted tree species in AFS can function as environmental filters, influencing the richness, density, and life-form composition of spontaneous vegetation. Although recruitment may also be influenced by propagule availability from surrounding vegetation, differences in the composition and abundance of planted species can generate contrasting local conditions for the establishment and persistence of spontaneous plants. Importantly, these life forms represent contrasting growth strategies and patterns of above- and belowground allocation. Herbaceous species generally concentrate biomass in shallow, rapidly cycling organs, whereas woody plants develop more persistent structural tissues and root systems. Consequently, shifts in the relative representation of herbs, shrubs, trees, palms, and lianas may be associated with contrasting patterns of fine- and coarse-root biomass during agroforestry development. Despite this potential linkage, spontaneous vegetation and root biomass have rarely been examined together as complementary components of early AFS development. This relationship is ecologically relevant because vegetation composition and structure can be associated with the development and spatial distribution of belowground biomass (Lima et al. 2020; Peng and Chen 2021). Therefore, examining how root biomass

covaries with the structure and life-form composition of spontaneous vegetation may provide insights into above–belowground associations during the early development of AFS established on degraded Amazonian lands. Importantly, these relationships may also depend on the ecological range considered. Young AFS occur within heterogeneous agricultural landscapes containing contrasting vegetation states, so associations between vegetation organization and belowground biomass may become more apparent across the broader landscape gradient than among recently established agroforestry configurations alone.

Fine roots, owing to their dynamic nature and rapid turnover, represent one of the most active components of belowground ecosystems and contribute to the primary productivity of terrestrial environments (Jackson et al. 1997; Gill and Jackson 2000). They play a significant role in soil carbon and nutrient cycling, regulating the input of organic matter through root exudation, respiration, and decomposition (Gill and Jackson 2000; Gordon and Jackson 2000; Valcarcel et al. 2007; Ratuchne et al. 2016). Coarser roots, in contrast, perform longer-lived structural, transport, and storage functions and therefore represent a distinct component of belowground biomass accumulation. Considering fine and coarse roots separately can thus provide complementary information on short-term resource-acquisition processes and longer-lived belowground structural development. The continuous death and renewal of fine roots contribute to organic-matter inputs and represent an important pathway linking vegetation dynamics to belowground carbon cycling (McCormack and Iversen 2019). In agroforestry systems, belowground biomass and its contribution to carbon cycling can vary with species composition, vegetation structure, and management practices (Ramos et al. 2017). These relationships reinforce the ecological importance of roots as a component connecting vegetation development with belowground ecosystem processes. However, comparatively little is known about how fine- and coarse-root biomass varies in relation to the composition and structure of spontaneous vegetation during the initial establishment of tropical agroforestry systems. This represents an important knowledge gap, particularly in degraded Amazonian landscapes where vegetation reorganization and belowground development occur simultaneously.

Understanding how spontaneous vegetation is associated with belowground biomass development can help clarify ecological processes in young agroforestry systems and inform vegetation management in smallholder landscapes. Given this context, this study evaluated variation in spontaneous vegetation and fine- and coarse-root biomass among five young agroforestry arrangements established on degraded pasture, using an adjacent secondary forest as a reference ecosystem, in Canaã dos Carajás, southeastern Pará, Brazil. Although the agroforestry arrangements shared major crop species, they differed in the identity and abundance of associated cultivated and woody species, providing distinct planted-community

configurations during the early stage of establishment. We addressed four complementary questions: (i) how do spontaneous-vegetation richness, density, life-form composition, and fine- and coarse-root biomass vary among the sampled AFS modules and relative to the secondary-forest reference? (ii) Are spontaneous-vegetation richness and density associated with fine- and coarse-root biomass across the broader ecological gradient encompassing the forest reference and AFS modules? (iii) Is life-form composition associated with root biomass across this forest–AFS gradient? and (iv) are these relationships also detectable when considering the young AFS modules alone? We expected the AFS modules and forest reference to differ in spontaneous-vegetation structure, life-form composition, and root biomass, reflecting their contrasting vegetation states. Across the complete forest–AFS gradient, we further expected root biomass to covary with spontaneous-vegetation richness, density, and life-form composition because herbaceous and woody life forms differ markedly in growth strategy and belowground organization. Given the early stage of AFS establishment and the comparatively narrower range of vegetation states represented by the AFS modules, we further evaluated whether these above–belowground relationships were also detectable within the agroforestry systems alone. To address these questions, we conducted field vegetation surveys and soil sampling across the five agroforestry modules and the adjacent secondary forest and quantified floristic composition, vegetation structure, and fine- and coarse-root biomass.

MATERIALS AND METHODS

Study Area

The study was conducted on a rural property located in the municipality of Canaã dos Carajás (6°16′46″ S, 49°50′56″ W), southeastern Pará, Brazil (Fig. 1). The regional climate is classified as "Aw" (tropical with a dry winter) according to Köppen's classification. The dry season occurs from May to October, while the rainy season extends from November to April. Mean annual precipitation is approximately 2,500 mm, and mean annual temperature is around 29 °C (Siqueira et al. 2012). The predominant soils in the region are dystrophic red-yellow Argisols, with frequent occurrences of dystrophic red-yellow Latosols and dystrophic Litholic Neosols (ICMBio 2016). The regional vegetation is predominantly represented by Amazonian forest formations, although the study property had previously undergone land-use conversion, and the area selected for AFS establishment consisted of degraded pasture adjacent to secondary forest.

On this property, five different AFS modules (M1–M5) were implemented, varying in species composition. The modules included cacao, banana, cassava, pineapple, and maize as common crops and differed in the identity and abundance of associated species (Table 1). Each

module covered approximately 2 ha, totaling 10 ha of demonstration area. All modules were established on the same property and shared the same previous land-use condition (degraded pasture), as well as similar topographic and edaphic conditions. This common environmental and land-use context reduced major sources of background variation and provided a consistent basis for comparing local patterns of vegetation and root biomass among the five agroforestry configurations. The modules nevertheless differed in the identity and abundance of associated cultivated and woody species (Table 1), providing distinct planted-community configurations within the same environmental setting. In addition to the AFS modules, an adjacent secondary forest area was used as a reference ecosystem (Oliveira et al. 2023).

Characterization of Spontaneous Vegetation

The floristic survey was carried out one year after AFS implementation. This sampling period was selected to characterize the early stage of vegetation development, when spontaneous vegetation was already established but the agroforestry systems were still undergoing rapid structural reorganization. Five 2 × 2 m plots were randomly established in each AFS module and in the secondary-forest reference area, totaling 30 plots and 120 m² of sampled area. Random placement of plots within each module was used to capture local spatial variation in spontaneous vegetation and reduce bias associated with subjective plot positioning. In each plot, all individuals ≤1.5 m in height were identified and counted. Plant material was processed as herbarium specimens and identified by specialists, following the APG IV classification system (The Brazil Flora Group et al. 2022). Species were classified into four life forms: herbs, lianas, trees/shrubs, and palms.

Root Biomass Sampling

To estimate root biomass, three soil samples were randomly collected in each plot at 0–10 cm depth using a stainless-steel cylinder (196 cm³), following the sampling approach previously applied to these agroforestry modules by Ribeiro et al. (2024). Sampling was restricted to the topsoil layer to characterize root biomass within the surface soil compartment, where interactions between roots and soil resources are particularly relevant (Lacerda et al. 2025). Samples were stored in plastic bags and refrigerated at 4 °C until processing.

Soil samples were washed under running water through a 0.5-mm mesh (Leão et al. 2014). Roots were manually separated and classified as fine (≤2 mm diameter) or coarse (>2 mm diameter) (Braga et al. 2017; Ramos et al. 2017). All roots recovered from the soil samples were included, irrespective of plant origin, and were classified by diameter rather than taxonomic identity. Thus, root biomass represented the belowground plant community

occurring within the sampled soil volume and could include roots from spontaneous vegetation, planted crops, and planted trees. Root samples were dried at 70 °C until constant weight and weighed on a precision analytical balance (0.0001 g). Root biomass was expressed as g m^{-2} (Braga et al. 2017). For each plot, fine- and coarse-root biomass values obtained from the three soil samples were averaged, resulting in a single value for each root-biomass fraction per plot.

Statistical Analysis

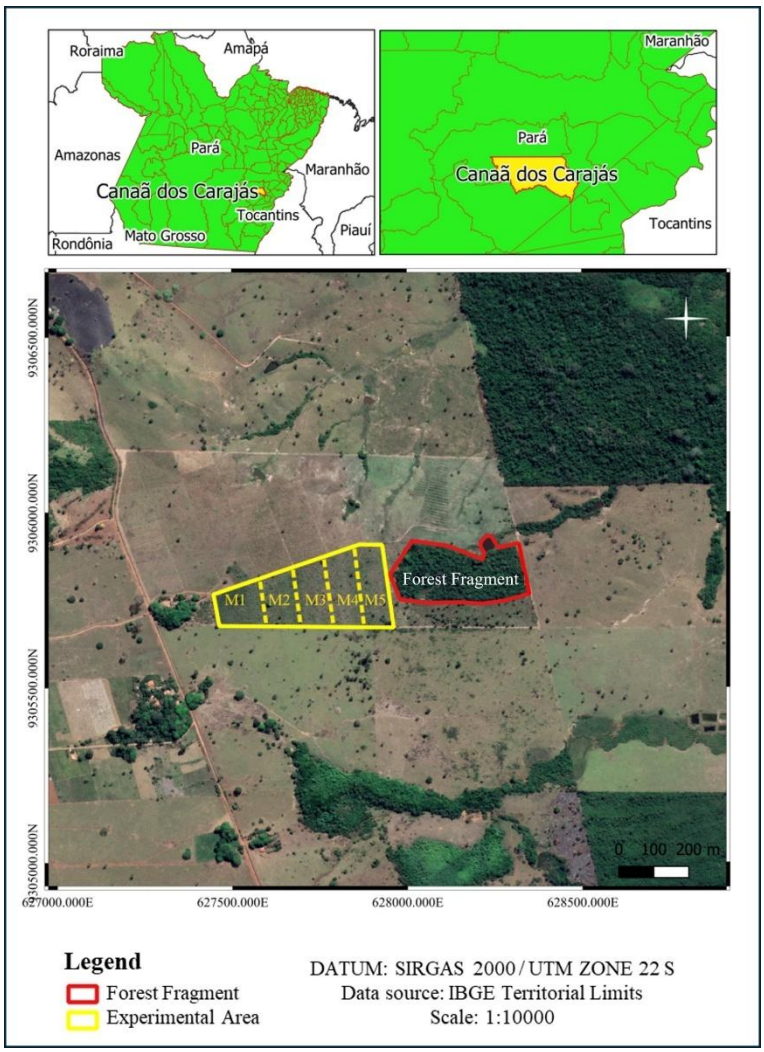
All statistical analyses were performed in R version 4.5.1 (R Core Team 2025). The sampling plot (2 × 2 m; $n = 30$) was considered the observational unit, with five plots sampled within each of the five AFS modules and the secondary-forest reference. The three soil samples collected within each plot were treated as subsamples, and their fine- and coarse-root biomass values were averaged before statistical analysis, resulting in a single value for each root-biomass fraction per plot. Because each agroforestry configuration was represented by a single module, plots within modules were considered spatial subsamples rather than independent replicates of the agroforestry arrangements. Accordingly, comparisons among modules characterize variation among the specific agroforestry configurations evaluated at the study site and were not interpreted as replicated experimental treatment effects.

Differences among AFS modules and the secondary-forest reference in species richness, individual abundance, and root biomass were evaluated using generalized linear models (GLMs). Species richness was modeled using a Poisson distribution with a log-link function. Individual abundance was initially modeled using a Poisson distribution, but substantial overdispersion was detected; therefore, a negative-binomial model with a log-link function was retained. Because all vegetation plots had the same area (4 m^2), model estimates for individual abundance were converted to individuals m^{-2} for graphical presentation and interpretation. Fine-root biomass was modeled using a Gamma distribution with a log-link function. Coarse-root biomass contained both zero values and positive continuous observations and was therefore modeled using a Tweedie distribution with a log-link function, which accommodates this distributional structure while retaining zero observations. Model adequacy was evaluated using residual diagnostics, including tests of residual uniformity, dispersion, and zero inflation when applicable. Estimated marginal means and pairwise contrasts among sampled areas were obtained using the *emmeans* package, with Tukey adjustment for multiple comparisons.

Associations between spontaneous-vegetation attributes and root biomass were examined using regression models fitted separately for fine- and coarse-root biomass, with species richness and individual abundance considered separately as continuous predictors. Fine-root biomass relationships were modeled using Gamma GLMs with a log-link function,

whereas coarse-root biomass relationships were modeled using Tweedie GLMs with a log-link function, retaining both zero and positive observations. Model-predicted relationships and 95% confidence intervals were used to characterize the direction and uncertainty of these associations. Analyses were first performed across the complete ecological gradient comprising the secondary-forest reference and all AFS modules. Complementary models were subsequently fitted using only AFS plots to determine whether relationships detected across the complete gradient were also evident within the agroforestry systems. These AFS-only models were treated as sensitivity analyses rather than replacements for the complete-gradient analyses.

The multivariate association between spontaneous-vegetation life-form composition and root biomass was evaluated using redundancy analysis (RDA) implemented in the *vegan* package. The explanatory matrix comprised the abundances of herbaceous plants, lianas, trees/shrubs, and palms at the plot level. Because these counts represent compositional information and vary with total abundance among plots, the life-form matrix was Hellinger-transformed before ordination. The response matrix comprised fine- and coarse-root biomass, which were standardized prior to analysis to place both root-biomass components on comparable scales. Statistical significance of the overall constrained model, individual canonical axes, and marginal contribution of each life-form variable was assessed using permutation tests with 9,999 permutations. Model fit was additionally quantified using adjusted R^2, which accounts for the number of explanatory variables relative to sample size and provides a less biased estimate of explained variation in constrained ordination (Peres-Neto et al. 2006). As with the univariate regressions, the RDA was first performed across the complete forest–AFS gradient and subsequently repeated using only AFS plots as a complementary sensitivity analysis. Relationships represented by the RDA were interpreted as multivariate associations rather than evidence of causal effects of spontaneous-vegetation life forms on root production.



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295 **Fig. 1** Location map of the study area showing the five agroforestry system modules (M1–M5)
296 in the municipality of Canaã dos Carajás, southeastern Pará, Brazil

Table 1 Species (scientific and common names), propagation methods, and number of propagules established in the five agroforestry system modules (M1–M5) in Canaã dos Carajás, southeastern Pará, Brazil. Species differing among modules are highlighted in bold

	Scientific name	Common name	Propagation method	Number of propagules
AFS-M1	*Musa* sp.	Banana	Seedling	1699
	Manihot esculenta Crantz	Cassava	Cutting	4600
	Ananas comosus (L.) Merr.	Pineapple	Seedling	18405
	Zea mays L.	Maize	Seed	10590
	***Cucurbita* sp.**	**Pumpkin**	Seed	833
	***Inga edulis* Mart.**	**Inga vine**	Seedling	50
	***Inga marginata* Willd.**	**Inga bean**	Seedling	25
	***Hymenaea* sp.**	**Jatoba**	Seedling	50
	***Cedrela* sp.**	**Cedar**	Seedling	15
	***Phaseolus vulgaris* L.**	**Bean**	Seed	5004
	***Carica papaya* L.**	**Papaya**	Seedling	350
	Theobroma cacao L.	Cocoa	Seedling	1583
			Total propagules	43204
AFS-M2	*Musa* sp.	Banana	Seedling	1603
	Manihot esculenta Crantz	Cassava	Cutting	6131
	Ananas comosus (L.) Merr.	Pineapple	Seedling	9203
	Zea mays L.	Maize	Seed	14116
	***Swietenia macrophylla* King**	**Mahogany**	Seedling	100
	***Cedrela* sp.**	**Cedar**	Seedling	25
	***Tamarindus indica* L.**	**Tamarind**	Seedling	15
	***Phaseolus vulgaris* L.**	**Bean**	Seed	5004
	Theobroma cacao L.	Cocoa	Seedling	2111
			Total propagules	38308
AFS-M3	*Musa* sp.	Banana	Seedling	1690
	Manihot esculenta Crantz	Cassava	Cutting	4600
	Ananas comosus (L.) Merr.	Pineapple	Seedling	9203
	Zea mays L.	Maize	Seed	10590
	***Inga edulis* Mart.**	**Inga vine**	Seedling	20
	***Copaifera* sp.**	**Copaiba**	Seedling	50
	***Dipteryx odorata* (Aubl.) Forsyth f.**	**Cumaru**	Seedling	20
	***Tamarindus indica* L.**	**Tamarind**	Seedling	50
	***Phaseolus vulgaris* L.**	**Bean**	Seed	5004
	Theobroma cacao L.	Cocoa	Seedling	1583
			Total propagules	32810
AFS-M4	*Musa* sp.	Banana	Seedling	1668
	Manihot esculenta Crantz	Cassava	Cutting	4600
	Ananas comosus (L.) Merr.	Pineapple	Seedling	9203
	Zea mays L.	Maize	Seed	10590
	***Hymenaea* sp.**	**Jatoba**	Seedling	50
	***Copaifera* sp.**	**Copaiba**	Seedling	50
	***Dipteryx odorata* (Aubl.) Forsyth f.**	**Cumaru**	Seedling	20
	***Cedrela* sp.**	**Cedar**	Seedling	40
	***Phaseolus vulgaris* L.**	**Bean**	Seed	5004
	Theobroma cacao L.	Cocoa	Seedling	1583
			Total propagules	32808
AFS-M5	*Musa* sp.	Banana	Seedling	1659
	Manihot esculenta Crantz	Cassava	Cutting	4600
	Ananas comosus (L.) Merr.	Pineapple	Seedling	9203
	Zea mays L.	Maize	Seed	10590
	***Cucurbita* sp.**	**Pumpkin**	Seed	833
	***Inga edulis* Mart.**	**Inga vine**	Seedling	25
	***Swietenia macrophylla* King**	**Mahogany**	Seedling	50
	***Copaifera* sp.**	**Copaiba**	Seedling	50
	***Cedrela* sp.**	**Cedar**	Seedling	40
	***Arachis hypogaea* L.**	**Peanut**	Seed	5004
	***Carica papaya* L.**	**Papaya**	Seedling	1668
	Theobroma cacao L.	Cocoa	Seedling	1583
			Total propagules	35305

RESULTS

A total of 5,048 individuals, representing 97 species and 35 botanical families, were recorded. Five families accounted for more than 80% of all individuals: Asteraceae (2,180 individuals), Phyllanthaceae (861), Euphorbiaceae (479), Fabaceae (277), and Linderniaceae (259). The most abundant species were *Synedrellopsis grisebachii* (1,911 individuals), *Phyllanthus niruri* (577), *Acalypha alopecuroides* (373), and *Phyllanthus urinaria* (280), which together accounted for 62% of all recorded individuals.

Mean species richness varied among the sampled areas, with the lowest values in FO and M1 (8.8 species per plot in both areas) and the highest value in M3 (16.2 species per plot). Significant pairwise differences were restricted to comparisons of M3 with FO and M1 (Tukey, $p = 0.014$ for both comparisons; Fig. 2a). Individual density was lower in FO (14.6 individuals m^{-2}) than in all AFS modules, where mean values ranged from 32.8 to 67.8 individuals m^{-2}. FO differed significantly from all AFS modules ($p < 0.05$), and M3 also had significantly greater density than M1 ($p = 0.024$; Fig. 2b).

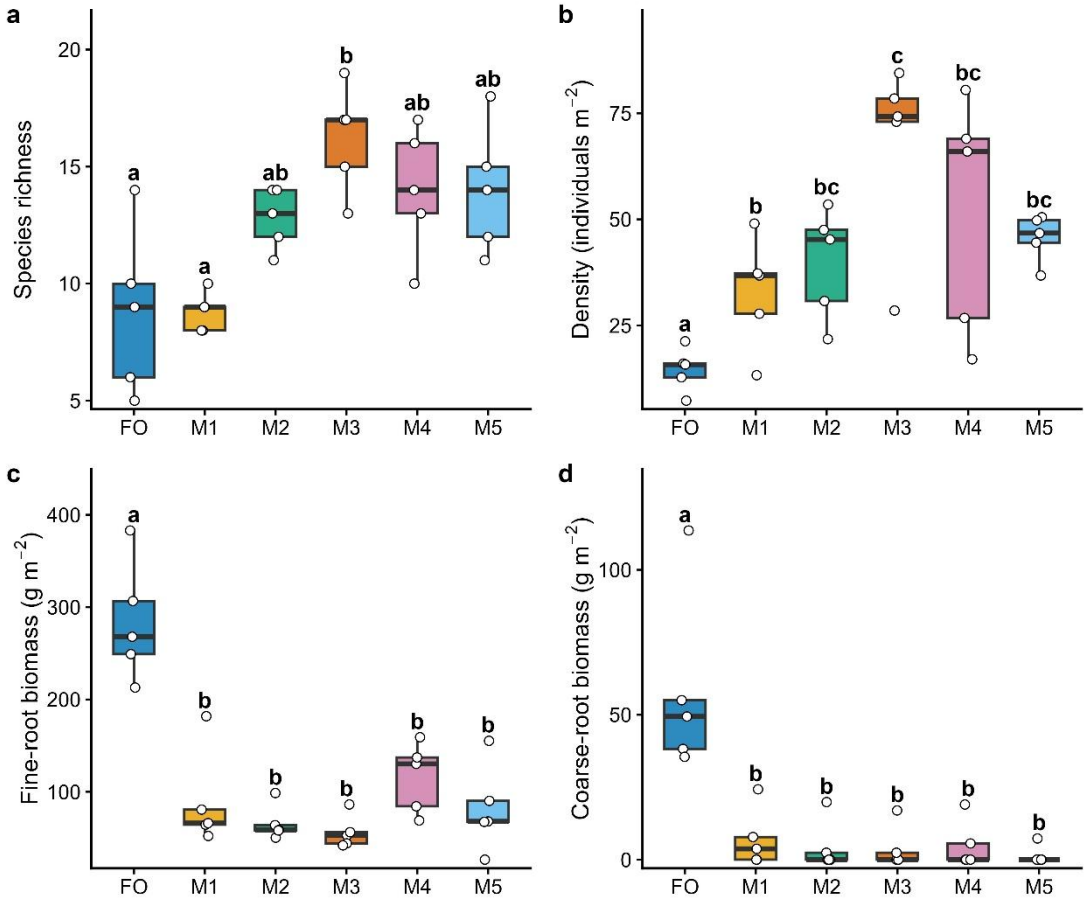
Herbaceous plants strongly predominated in all AFS modules, accounting for 88.2–97.1% of the recorded individuals. In contrast, FO was dominated by trees/shrubs (52.1%), followed by herbaceous plants (41.4%). Lianas represented a small proportion of individuals across all sampled areas, whereas palms accounted for 4.8% of individuals in FO and were nearly absent from the AFS modules (Fig. 3).

Root biomass showed a contrasting pattern. Fine-root biomass was significantly higher in FO (284.0 g m^{-2}) than in any AFS module (56.3–116.0 g m^{-2}; $p < 0.05$), whereas no significant differences were detected among AFS modules (Fig. 2c). Coarse-root biomass followed a similar pattern, being markedly greater in FO (58.4 g m^{-2}) than in the AFS modules (1.5–7.2 g m^{-2}; $p < 0.001$). FO differed significantly from all AFS modules, whereas no significant differences in coarse-root biomass were detected among M1–M5 (Fig. 2d).

Across the complete ecological gradient encompassing the forest reference and the AFS modules, both fine- and coarse-root biomass were negatively associated with spontaneous-vegetation attributes (Fig. 4). Fine-root biomass decreased with increasing species richness ($p = 0.006$; Fig. 4a) and number of individuals ($p = 0.010$; Fig. 4b). Coarse-root biomass showed similar negative relationships with species richness ($p = 0.017$; Fig. 4c) and number of individuals ($p < 0.001$; Fig. 4d). Forest plots generally occupied the portion of these gradients characterized by lower richness and abundance of the surveyed spontaneous vegetation and greater root biomass, whereas AFS plots generally showed greater richness and abundance and lower root biomass.

Life-form composition was significantly associated with root biomass across the complete forest–AFS gradient (Fig. 5). The RDA was significant ($p < 0.001$) and accounted for a substantial proportion of the multivariate variation (adjusted $R^2 = 0.66$). RDA1 accounted for 67.9% of the total variation and RDA2 for 2.5%. Herbaceous vegetation was oriented toward the positive side of RDA1 and opposite to the vectors representing fine- and coarse-root biomass. Trees/shrubs and palms were positioned toward the negative side of RDA1, broadly in the same direction as the root-biomass vectors, whereas lianas were primarily oriented along RDA2. FO plots occurred predominantly toward the negative side of RDA1, associated with greater root biomass and greater relative representation of trees/shrubs and palms, whereas most AFS plots were concentrated toward the positive side of RDA1, associated with herbaceous dominance.

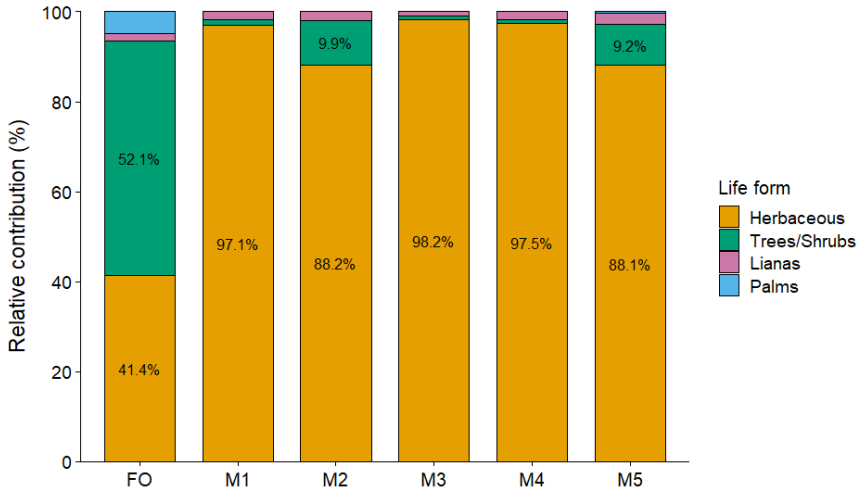
When the analyses were restricted to the AFS modules, however, the relationships detected across the complete forest–AFS gradient were no longer evident (Fig. S1). Fine-root biomass was not significantly associated with species richness ($p = 0.427$; Fig. S1a) or number of individuals ($p = 0.553$; Fig. S1b). Likewise, coarse-root biomass was not significantly associated with species richness ($p = 0.733$; Fig. S1c) or number of individuals ($p = 0.178$; Fig. S1d). Consistent with these results, life-form composition was not significantly associated with root biomass when only AFS plots were considered (RDA, $p = 0.993$; adjusted $R^2 = -0.16$; Fig. S2), with RDA1 and RDA2 accounting for only 2.6% and 0.9% of the total variation, respectively. Thus, the relationships between spontaneous-vegetation attributes, life-form composition, and root biomass detected across the complete forest–AFS gradient were not detected within the young AFS modules alone.



357

358 **Fig. 2** Species richness (a), individual density (b), fine-root biomass (c), and coarse-root
359 biomass (d) in the forest reference (FO) and agroforestry system modules (M1–M5) in Canaã
360 dos Carajás, southeastern Pará, Brazil. Different lowercase letters indicate significant
361 differences among sampled areas based on Tukey-adjusted pairwise comparisons ($p < 0.05$)

362



363

364 **Fig. 3** Relative contribution of vegetation life forms to the spontaneous plant community
365 surveyed at ≤1.5 m in height in the forest reference (FO) and agroforestry system modules (M1–
366 M5) in Canaã dos Carajás, southeastern Pará, Brazil. Percentages were calculated from the total
367 number of individuals recorded for each life form within each sampled area

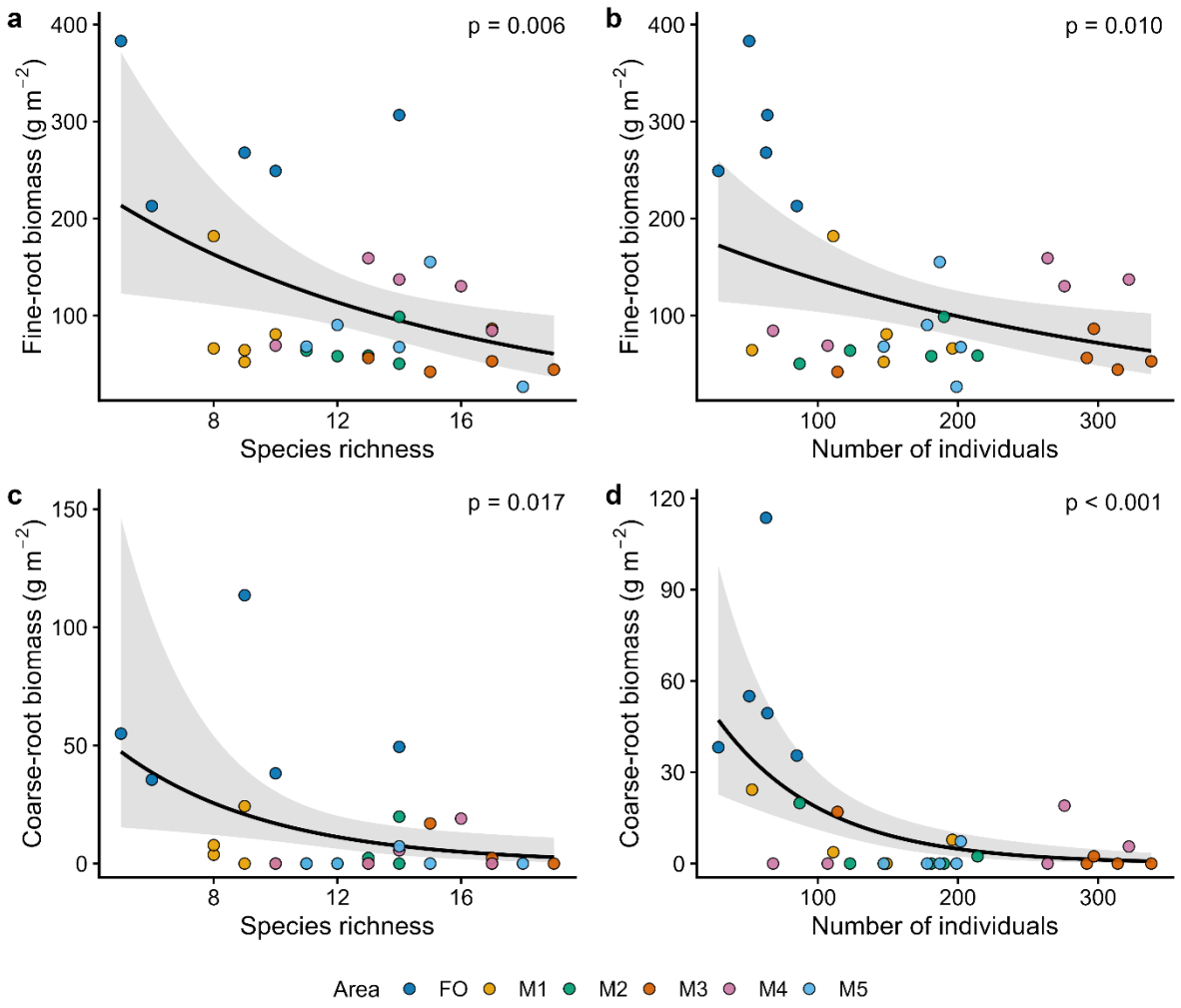


Fig. 4 Relationships of fine-root biomass with species richness (a) and number of individuals (b), and of coarse-root biomass with species richness (c) and number of individuals (d), across the forest reference (FO) and agroforestry system modules (M1–M5) in Canaã dos Carajás, southeastern Pará, Brazil. Lines represent model-predicted relationships, and shaded areas indicate 95% confidence intervals

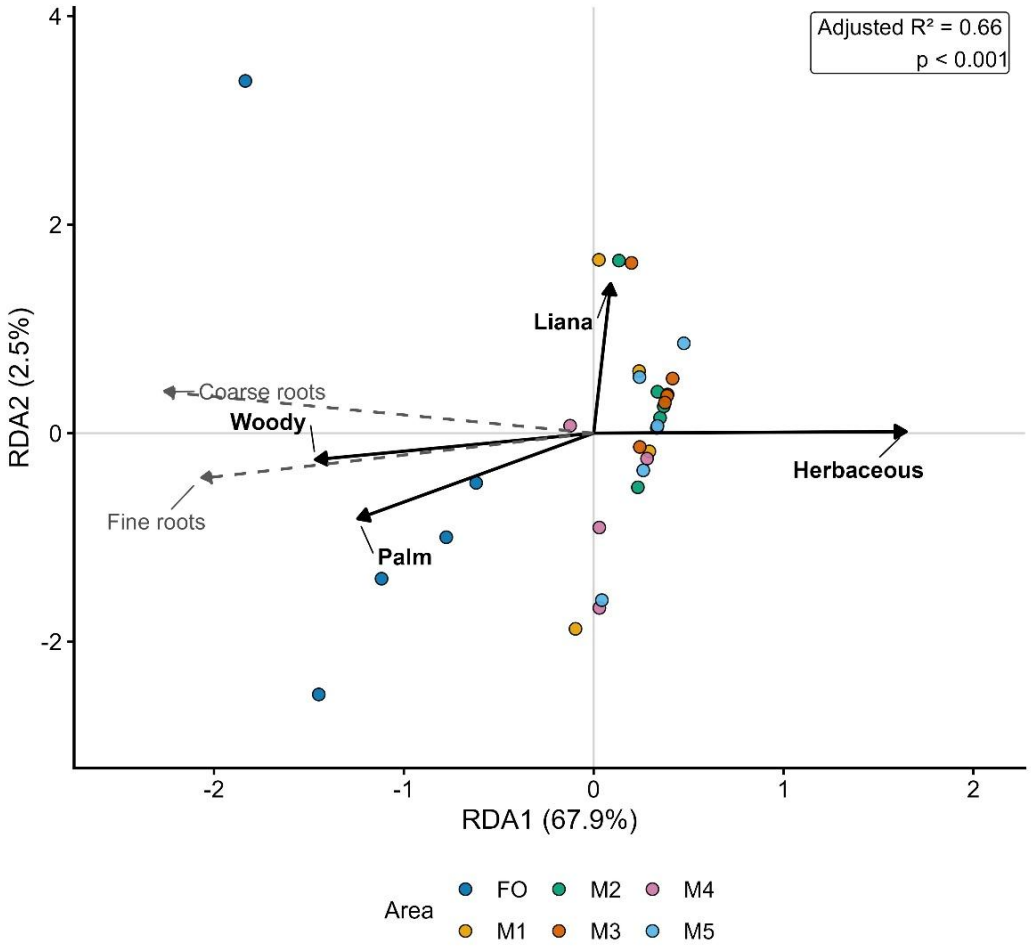


Fig. 5 Redundancy analysis (RDA) showing the association between vegetation life-form composition and fine- and coarse-root biomass across the forest reference (FO) and agroforestry system modules (M1–M5) in Canaã dos Carajás, southeastern Pará, Brazil. Solid vectors represent vegetation life forms, and dashed vectors represent root-biomass variables. Life-form abundances were Hellinger-transformed before analysis. RDA1 and RDA2 accounted for 67.9% and 2.5% of the total variation, respectively (adjusted $R^2 = 0.66$; $p < 0.001$)

DISCUSSION

Our expectations were partially supported, revealing a clear contrast between above- and belowground patterns during early agroforestry development. Only one year after AFS establishment, spontaneous vegetation already differed among modules in species richness and individual density, while life-form composition distinguished the herbaceous-dominated AFS from the more woody secondary-forest reference. Belowground biomass showed a different pattern: fine- and coarse-root biomass remained comparatively similar among AFS modules but were substantially greater in the forest reference. Across the complete forest–AFS gradient, spontaneous-vegetation richness and abundance were negatively associated with both root-biomass fractions, and life-form composition was strongly associated with their variation. Importantly, however, none of these relationships remained detectable when the analyses were restricted to the young AFS. Together, these findings reveal two complementary features of early agroforestry development: spontaneous vegetation can differentiate among young agroforestry configurations before comparable differences become apparent in belowground biomass, and apparent above–belowground associations depend strongly on the ecological range considered. Relationships emerging across the broader forest–AFS mosaic therefore should not be interpreted as evidence of processes already operating among young AFS configurations. This distinction is particularly relevant for smallholder agroforestry landscapes, where spontaneous vegetation may provide an early signal of ecological reorganization, whereas belowground differentiation may require longer-term monitoring to become detectable. The marked differences in spontaneous-vegetation richness and density among AFS modules indicate that naturally recruiting vegetation can respond rapidly during the early stages of system development. Rather than representing a passive component of these systems, spontaneous vegetation may reflect differences in planted-community structure, resource availability, management, and local recruitment opportunities (Lacerda et al. 2013; dos Santos et al. 2021; Oliveira et al. 2023). Within this broader pattern, M1 exhibited relatively low species richness and density, which may be related to the greater number and abundance of intercropped agronomic and forestry species introduced into this module (Table 1). As noted by Oliveira et al. (2023), greater occupation by cultivated species, combined with intensive management, can restrict opportunities for spontaneous plant establishment. Greater occupation of aboveground space by cultivated vegetation may reduce light availability and alter microsite conditions for recruitment, while shading and litter deposition can suppress the germination or establishment of light-demanding spontaneous species common in agroecosystems (Gomes and Christoffoleti 2008). These mechanisms provide plausible explanations for the differences observed among modules but cannot be directly attributed to the planted arrangements because

canopy cover, light availability, litter accumulation, and resource competition were not measured. Moreover, spontaneous recruitment is unlikely to depend exclusively on local conditions within each AFS. Studies in rural mosaics of the Eastern Amazon have shown that plant richness and regeneration can also be influenced by the composition of surrounding land uses, forest cover, and the availability of propagule sources at the landscape scale (Do Vale et al. 2017, 2018). Thus, the lower richness and density observed in M1 should be interpreted as an ecological pattern associated with its particular configuration and landscape context rather than as a replicated treatment effect.

At the community level, the 97 spontaneous species recorded across the sampled systems further demonstrate that young AFS established on degraded pasture can harbor substantial naturally recruiting plant diversity. Diverse spontaneous floras have also been documented in agroforestry systems across Pará, including systems in Tomé-Açu, Oeiras, and Capitão-Poço (Lacerda et al. 2013; Bolfe and Batistella 2011; Alves Filho et al. 2020; Santos and Costa 2021). However, direct comparisons of absolute species richness among these studies should be interpreted cautiously because sampling effort, AFS age, planted composition, management intensity, surrounding vegetation, and land-use history differ among sites. This context dependence is consistent with evidence from rural landscapes in the Eastern Amazon showing that plant richness is influenced not only by local land use but also by the composition of land-use types within the surrounding mosaic (Do Vale et al. 2017). More recent evidence further indicates that beta diversity among land-use types makes a major contribution to plant diversity in Eastern Amazonian agricultural mosaics and that the ecological quality of the environmental matrix is particularly important for conservation (Silva et al. 2024). These findings reinforce the importance of considering spontaneous vegetation in AFS within the broader landscape mosaic rather than interpreting each productive unit as an ecologically isolated system.

The strong predominance of herbaceous plants in the AFS modules (88.2–97.1% of individuals), compared with the greater representation of trees/shrubs in the forest reference, further emphasizes the contrasting vegetation states represented within the sampled landscape. Herbaceous dominance is common in recently disturbed and managed environments because many species possess ecological strategies such as rapid growth, prolific seed production, seed dormancy, and persistent soil seed banks (Costa and Mitja 2009; Lacerda et al. 2013, 2016). This pattern is also consistent with observations from agricultural mosaics in southeastern Pará, where pioneer herbs are associated with early successional stages and agricultural land uses characterized by high irradiance, altered soils, competition, and recurrent disturbance (Do Vale et al. 2018). By contrast, the greater representation of woody life forms and palms in the

secondary forest is consistent with a more structurally developed vegetation state. The occurrence of spontaneously recruiting trees, shrubs, and palms within young AFS is nevertheless ecologically relevant because these groups represent potential components of natural regeneration and may contribute progressively to structural complexity as agroforestry systems develop (Vieira and Proctor 2007; Lacerda et al. 2016). The lower vegetation stratum is itself an important component of regeneration dynamics, whereas forested portions of rural mosaics can act as sources of seeds and propagules for surrounding land uses (Do Vale et al. 2017). Their presence should therefore not necessarily be viewed as undesirable competition with cultivated plants, particularly where management objectives include ecological restoration, diversification, and increased structural complexity.

In contrast to spontaneous vegetation, fine- and coarse-root biomass did not differ significantly among AFS modules. All modules were, however, clearly differentiated from the secondary-forest reference, which contained substantially greater fine- and coarse-root biomass. This contrast is ecologically consistent with the greater structural development and woody component of the forest reference compared with agroforestry systems sampled only one year after establishment. Forest vegetation maintains established root systems associated with multiple vegetation strata and a longer period of belowground development, whereas the young AFS were established on degraded pasture and remained in an early phase of vegetation reorganization. Fine-root biomass can vary with vegetation type, successional stage, and land-use history, and its reorganization following disturbance may occur over relatively long timescales. In an Eastern Amazonian terra firme forest, Leão et al. (2014) found that very fine- and fine-root biomass had not significantly recovered even 14 years after selective logging, highlighting the potentially long timescale of belowground responses following vegetation disturbance. The relatively narrow range of root biomass among AFS modules therefore suggests that differences in their planted-community configurations had not yet translated into detectable differences in belowground biomass at the sampled depth. Their common previous land use and similar environmental setting may also have contributed to this pattern. Previous research across contrasting land-cover types in the Amazonian Arc of Deforestation has further demonstrated that fine-root biomass varies with land cover and that its multivariate structure shares substantial variation with vegetation structure (Lima et al. 2020). Together, these findings support the interpretation that belowground biomass is related to vegetation development and land-cover state, while also indicating that its response to changes in vegetation structure may occur over longer timescales than the early differentiation observed in spontaneous vegetation. Rather than indicating that planted configuration is unimportant for belowground development, the absence of significant differences among the five AFS modules

suggests that one year of establishment may have been insufficient for differences among these young agroforestry configurations to become clearly expressed in root biomass.

The negative relationships between spontaneous-vegetation richness and abundance and both fine- and coarse-root biomass across the complete forest–AFS gradient initially appear contrary to the positive diversity–biomass relationships often attributed to niche complementarity (Meinen et al. 2009; Brassard et al. 2013). However, the AFS-only analyses substantially clarify the ecological meaning of this result. None of these relationships remained significant when the secondary-forest reference was excluded. Therefore, the negative associations should not be interpreted as evidence that increasing spontaneous-plant richness or abundance reduces root biomass, nor as evidence for a particular biomass-allocation mechanism. Instead, they characterize a broader ecological gradient in which forest plots combined greater root biomass with lower richness and abundance of the vegetation surveyed at ≤1.5 m, whereas young AFS combined abundant herbaceous recruitment with comparatively low root biomass. The absence of these relationships within the AFS modules does not invalidate the broader-gradient associations; rather, it indicates that the relationship depends on the ecological range considered and becomes evident when contrasting vegetation states within the landscape are analyzed together. This distinction is especially important because the vegetation survey characterized spontaneous individuals ≤1.5 m in height, whereas root samples integrated all roots present in the sampled soil volume.

This dependence on ecological range is meaningful in heterogeneous Amazonian agricultural landscapes. Agroforestry systems do not function as isolated ecological units but occur within heterogeneous landscape mosaics composed of cultivated areas, pastures, secondary vegetation, and forest remnants. This interpretation is particularly relevant to the present study area. Previous sampling conducted in the same landscape, including the same five AFS modules and the adjacent secondary forest and pasture, showed pronounced differences in fine-root biomass among land-cover states, while the five young AFS modules remained comparatively similar. Notably, both the secondary forest and the adjacent *Urochloa* pasture supported substantially greater fine-root biomass than the young AFS, despite their contrasting vegetation structure (Ribeiro et al. 2024). This finding indicates that belowground biomass across this landscape cannot be interpreted simply as a linear response to increasing woody vegetation; rather, contrasting land-cover states may support distinct belowground configurations associated with their vegetation structure, dominant life forms, land-use history, and management. Studies conducted in Eastern Amazonian rural mosaics have further demonstrated that land-use history, forest cover, matrix characteristics, and landscape connectivity influence vegetation composition, recruitment, and successional trajectories (Do

Vale et al. 2017, 2018). Furthermore, habitat heterogeneity and differences among land-use types make substantial contributions to landscape-level plant diversity, while the quality of the surrounding environmental matrix influences conservation value (Silva et al. 2024). Consequently, ecological relationships that are weak or undetectable within the comparatively narrow range represented by young AFS may become more clearly expressed when the broader heterogeneity of vegetation states within the agricultural landscape is considered. Our complete-gradient analyses therefore address a broader ecological contrast within this heterogeneous landscape, whereas the AFS-only analyses address whether the same relationships are already detectable among recently established agroforestry configurations.

The multivariate analysis reinforces this interpretation. Across the complete forest–AFS gradient, life-form composition was strongly associated with root biomass, with the RDA accounting for a substantial proportion of the multivariate variation (adjusted $R^2 = 0.66$; $p < 0.001$). Herbaceous dominance characterized most AFS plots along the positive side of RDA1, whereas greater root biomass and greater representation of trees/shrubs and palms characterized the opposite side of the ordination. This correspondence between aboveground vegetation organization and belowground biomass is consistent with previous evidence from contrasting land-cover types in the Amazonian Arc of Deforestation, where vegetation structure shared a substantial component of multivariate variation with fine-root biomass (Lima et al. 2020). Thus, the substantial multivariate association observed across our complete gradient is ecologically coherent with broader regional evidence that belowground biomass varies in association with vegetation structure across heterogeneous land-cover states. However, this multivariate association was absent when only AFS plots were analyzed (adjusted $R^2 = -0.16$; $p = 0.993$). Taken together, these results suggest that vegetation life-form composition becomes informative for understanding belowground biomass primarily across the broader range of vegetation structures represented in the forest–AFS mosaic, whereas the young AFS alone currently occupy a narrower range of above- and belowground conditions.

This interpretation must nevertheless account for the different biological compartments represented by the vegetation and root datasets. Roots were classified by diameter rather than taxonomic origin and may therefore have originated from spontaneous vegetation, planted crops, planted trees, or other vegetation whose roots reached the sampled soil volume. Consequently, the correspondence between spontaneous-vegetation life-form composition and root biomass represents ecosystem-level covariance rather than evidence that the spontaneous plants recorded aboveground directly produced the sampled roots. This limitation is particularly relevant for the forest reference, where the survey of individuals ≤1.5 m characterized the lower vegetation stratum but the sampled roots integrated contributions from the entire vegetation

community. The results therefore support an association between vegetation organization and belowground biomass across ecological states, but not direct attribution of particular root fractions to individual life forms.

Importantly, the contrast between the secondary forest and young AFS should not be viewed merely as a methodological artifact or as a comparison between unrelated land-use categories. In Amazonian agricultural landscapes, productive areas, secondary vegetation, and forest remnants coexist as components of the same landscape matrix. Evidence from Eastern Amazonian agricultural mosaics demonstrates that the amount and conservation condition of forest fragments can influence propagule dispersal and successional development in surrounding land uses (Do Vale et al. 2018), while mature and secondary forests make particularly important contributions to the conservation of plant diversity within agricultural mosaics (Silva et al. 2024). The adjacent secondary forest therefore provides an ecologically meaningful reference ecosystem for evaluating how recently established agroforestry systems differ from more structurally developed forest vegetation. At the same time, its inclusion allows the study to capture part of the ecological heterogeneity that characterizes productive Amazonian landscapes.

Fine roots remain particularly important in this context because their rapid turnover contributes to nutrient cycling and organic-matter inputs, whereas coarse roots represent more persistent structural components of belowground biomass (Gill and Jackson 2000; McCormack and Iversen 2019; Weemstra et al. 2020). Our results therefore have implications for understanding the distribution of belowground plant biomass—and consequently the vegetation component of belowground carbon pools—across contrasting vegetation states. However, because soil carbon stocks, root production, turnover rates, and taxonomic root origin were not measured, the present results should not be interpreted as direct evidence of soil carbon sequestration or differential carbon allocation among spontaneous life forms. Instead, root biomass provides a snapshot of belowground vegetation structure during an early stage of agroforestry establishment. Long-term monitoring will be necessary to determine whether differences in planted-community composition and spontaneous vegetation eventually translate into divergent fine- and coarse-root biomass as the AFS mature.

From a management perspective, the results indicate that spontaneous vegetation should not be considered solely as a competing component to be eliminated from agroforestry systems. Its high richness, rapid establishment, and inclusion of spontaneously recruiting woody species indicate potential roles in soil protection, natural regeneration, habitat provision, and increasing vegetation complexity (Marshall et al. 2003; Lacerda et al. 2016; Kinyili et al. 2024). Management should therefore seek a balance between controlling excessive competition with

cultivated species and retaining spontaneous components that contribute to ecological functions and successional development. More broadly, our results support viewing agroforestry systems and forest remnants as complementary components of heterogeneous productive landscapes rather than as ecologically independent land uses. Maintaining forest remnants while promoting structurally diverse agroforestry systems may contribute to maintaining propagule sources, natural regeneration, and vegetation complexity within productive landscapes while sustaining agricultural production. Future studies incorporating replicated agroforestry configurations, measurements of canopy structure and soil properties, spatial relationships with surrounding forest remnants, taxonomic or molecular identification of roots, and repeated sampling through time will be particularly valuable for determining how these above- and belowground components become coupled as agroforestry systems mature.

CONCLUSION

In young agroforestry systems, spontaneous vegetation showed early differentiation in species richness and density, whereas fine- and coarse-root biomass remained similar among AFS modules one year after establishment. The secondary-forest reference, in contrast, supported substantially greater root biomass and a greater representation of woody life forms. Across the broader forest–AFS gradient, spontaneous-vegetation attributes, particularly life-form composition, were associated with root biomass, whereas these relationships were not detected within the young AFS alone. This dependence on the ecological range considered indicates that relationships between vegetation organization and belowground biomass become more clearly expressed when the broader heterogeneity of the forest–agroforestry mosaic is considered, and should not be extrapolated to relationships among young AFS configurations. Because root taxonomic identity was not determined, these associations represent ecosystem-level covariance rather than direct contributions of particular spontaneous life forms. For smallholder agroforestry landscapes, our findings suggest that spontaneous vegetation can provide an early signal of ecological reorganization, whereas belowground differentiation may require longer-term monitoring to become detectable. Recognizing spontaneous vegetation as part of this recovery process, rather than solely as vegetation to be suppressed, may help inform management strategies that reconcile agricultural production with natural regeneration and increasing vegetation complexity in heterogeneous Amazonian landscapes.

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Author contributions

TGS contributed to data collection, data analysis, and manuscript preparation. JAHR, LHDA, AJPC, NMM, and ABS contributed to field data collection and laboratory sample processing. PMV contributed to manuscript review and scientific interpretation. FCBL conceptualized and supervised the research and contributed to study design, field coordination, statistical analysis, and manuscript revision. All authors reviewed and approved the final version of the manuscript.

Statements and Declarations

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Competing interests

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

Data availability

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

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