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RESEARCH ARTICLE

Microbiome resilience of Amazonian forests: Agroforest divergence to bacteria and secondary forest succession convergence to fungi

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Abstract

An alarming and increasing deforestation rate threatens Amazon tropical ecosystems and subsequent degradation due to frequent fires. Agroforestry systems (AFS) may offer a sustainable alternative, reportedly mimicking the plant–soil interactions of the natural mature forest (MF). However, the role of microbial community in tropical AFS remains largely unknown. This knowledge is crucial for evaluating the sustainability of AFS and practices given the key role of microbes in the aboveground–belowground interactions. The current study, by comparing different AFS and successions of secondary and MFs, showed that AFS fostered distinct groups of bacterial community, diverging from the MFs, likely a result of management practices while secondary forests converged to the same soil microbiome found in the MF, by favoring the same groups of fungi. Model simulations reveal that AFS would require profound changes in aboveground biomass and in soil factors to reach the same microbiome found in MFs. In summary, AFS practices did not result in ecosystems mimicking natural forest plant–soil interactions but rather reshaped the ecosystem to a completely different relation between aboveground biomass, soil abiotic properties, and the soil microbiome.

KEYWORDS
aboveground–belowground interactions, forest regrowth, homegarden, land-use changes, slash-and-burn agriculture, soil microbiome, tropical rainforest

1 | INTRODUCTION

Over the past decade Amazonian rainforest has been converted to commodity production (pasture and soybean) at a rate of 6.54 M hectares per year (Kim et al., 2015). To circumvent the limitations presented by nutrient-poor soils, many farmers adopt slash-and-burn practices, which use fire to quickly mineralize nutrients stored in the plant biomass and make them available for subsequent crops.

However, the soils of the humid tropics are particularly vulnerable to degradation as the warm and humid environment promotes rapid organic matter decomposition and mineralization, nutrient loss caused by leaching and runoff (Markewitz et al., 2004), and gaseous nitrogen losses (Brookshire et al., 2017). Production thus declines rapidly after burning, causing farmers to abandon such land and move to a different plot of the forest, leading to further deforestation. Ultimately, repeated slash-and-burn cycles and shortened fallow periods (Lawrence et al., 2010) lead to reduced agronomic productivity (Runyan et al., 2012; Styger et al., 2007), thereby exacerbating rural poverty (Jakovac et al., 2016; Satyam Verma, 2012).

Agroforestry has been proposed a sustainable alternative to slash-and-burn shifting cultivation in the tropics. The core principle of agroforestry systems (AFS) lies in combining trees with crops, and/or animals in the same plot of land (a multistrata system) (Atangana et al., 2014) to mimic plant succession in the spontaneous forest (Cezar et al., 2015; Young, 2017), while including crop production (Cardozo et al., 2015, 2022). When appropriately managed, agroforestry practices improve the topsoil physico-chemical properties by increasing phosphorus and potassium contents (Pinho et al., 2012), maintain soil organic matter content (Leite et al., 2014), and promote nutrient cycling via nutrient pumping and safety net mechanisms (Seneviratne et al., 2006), which all strictly depend on ecosystem services delivered by the soil microbes (Wagg et al., 2014). Therefore, integrating the soil microbial community with the aboveground biomass and soil factors provides a fuller overview of the impacts of different management practices on the aboveground–belowground interactions in AFS.

Intentionally or unintentionally, AFS are designed to spatially, physically, and temporally optimize resource use by maximizing the positive interactions, and minimizing the negative interactions between plants and soil subsystems (aboveground–belowground interactions). However, compared with the spontaneous forests, agroforestry weakens the intensity of aboveground (plant)–belowground (soil chemical factors) (Leite et al., 2016). Thus, while the non-sustainable land use intensification in slash-and-burn practices clearly has negative impacts on soil nutrient recycling, above- and belowground biodiversity and ecosystem functioning and stability (Thiele-Bruhn et al., 2012), intensely managed AFS may likewise interfere in the aboveground–belowground linkages that impact ecosystem functioning, especially nutrient cycling.

The challenge in investigating the aboveground–belowground interactions in an agroforestry system begins with the multiple components or subsystems that play a major role in determining system functioning. Research on Amazonian forests to date has generally focused on tree–crop interactions (González & Kröger, 2020; Maezumi et al., 2018; Pinho et al., 2011, 2012; Stabile et al., 2020) or plant–animal interactions, and few studies in other tropical regions (Africa, Central America, and Asia) considered the impact of agroforestry practices on soil microorganisms (Liu et al., 2019; Schneider et al., 2015; Wemheuer et al., 2020). To our knowledge, no studies have considered the interaction between above- and belowground in a holistic approach including soil microbiome, the main players in soil nutrient cycling, in AFS and compared these systems with the secondary succession and mature Amazonian rainforests. Hence, here we investigated the capacity of the AFS to mimic the aboveground–belowground interactions found in mature forests (MFs) and compare that with spontaneous secondary forest recovery. We linked microbiome features to measures of aboveground vegetation biomass, litter mass, and the topsoil physico-chemical properties. By including the soil microbiome, we contribute to the design of more sustainable systems that better mimic the aboveground–belowground interactions of MFs.

2 | MATERIALS AND METHODS

2.1 | Field survey, site selection, and classification

The study was conducted in the eastern periphery of Amazonia, on 56 study sites in six counties (Anajatuba–Itinga, Arari, Morros–Rosário, São Luís, Gurupi, and Tomé-Açu), 40 of the 56 sites were located in central-northern Maranhão state, the others were approximately 400 km further westward in Tomé-Açu county in the eastern Pará state, Brazil (Figure 1). The maximum distance between sites within each county was <30 km, and the maximum distance between counties within each regional cluster was <150 km. According to the Köppen classification, climate is *Aw* and *Ami*, and varies slightly between the two regional clusters (2100 mm annual rainfall in central Maranhão state and 2300 mm in eastern Pará state, with 6 and 5 months of hydric deficit, respectively). Soils are nutrient-poor acid Oxisols or Ultisols (USDA, 2010), and the topsoil texture is loamy/fine sand.

We classify and compare four types of spontaneous forests with three types of planted or partially planted agroforests. We cover spontaneous secondary forest succession in young, mid-age and old spontaneous secondary forests and mature rainforest, and compare these with three types of agroforests (enriched fallow agroforest; homegarden agroforest; commercial plantation agroforest). Site selection and classification was based on the work of Cardozo et al. (2015) and Leite et al. (2016), as follows:

(i) Spontaneous secondary and mature rainforests: Secondary forests following slash-and-burn shifting cultivation or on abandoned pastures. Young secondary forests (YSF) consisted of sites that were recently (5–12 years ago, five sites) to slash-and-burn agriculture (Pollini, 2014). Mid-age secondary forests (MSF) represented sites where the last cycle of slash-and-burn agriculture occurred 15–20 years ago (six sites). Old secondary forests (OSF) grouped the sites reportedly in a fallow period of more than 30 years (seven sites). Mature forests (12 sites) were also distinguished, and indicated original MFs without any visible human perturbation or with low-intensity selective logging >60 years ago.

(ii) Agroforests: We distinguish in three types of AFS with contrasting structure and management: enriched fallow agroforests (EFAs, six sites), established by enrichment planting of fruit and timber species in the understory of 15–25-year-OSFs; homegarden

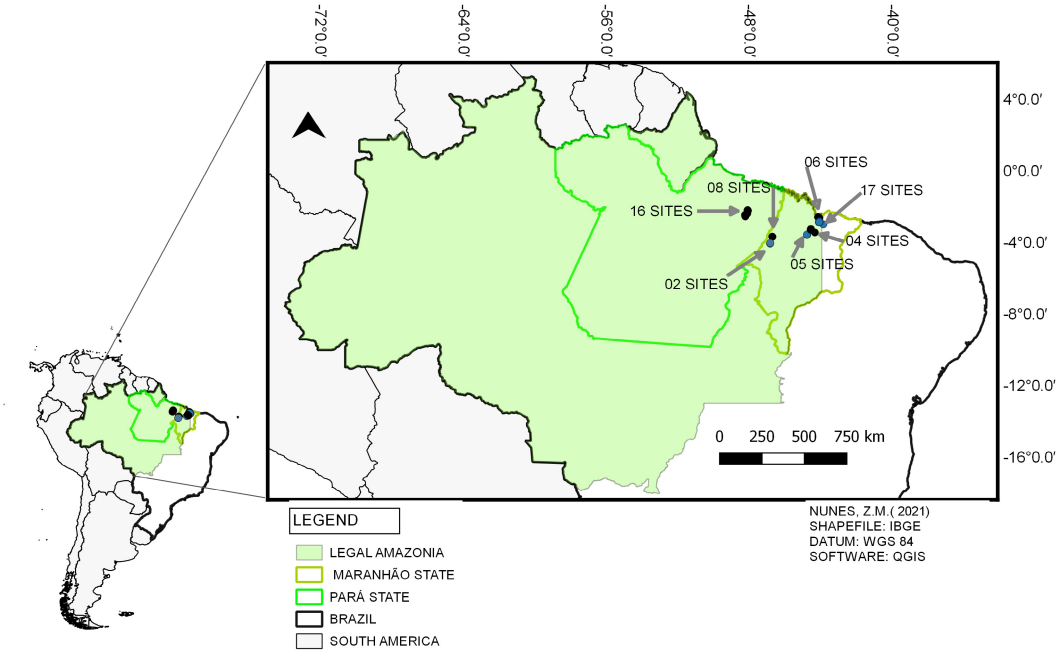

FIGURE 1 Map of the sampling sites within the Amazon eastern periphery. Map lines delineate the study areas and do not necessarily depict accepted national boundaries.

agroforests (HAs, 13 sites), tall multistrata agroforests surrounding houses, virtually omnipresent in the study region and throughout the tropics (Kumar & Nair, 2004); and commercial plantation agroforests (CPAs, seven sites), regularly spaced plantations with inorganic fertilization and liming, elaborated or inspired by Japanese immigrants. Only CPA had received fertilization (NPK applied close to the plants and following the agronomic recommendations of each species) as well as initial liming. According to Cardozo et al. (2022), the most common species in the agroforests were: açaí (*Euterpe oleracea* Mart.), mango (*Mangifera indica* L.), banana (*Musa* spp.), cupuassu (*Theobroma grandifolium* Wild ex. Spreng), cocoa (*Theobroma cacao* L.), and cashew (*Anacardium occidentale* L.). Table S1 classifies our 56 study sites according to their land-use and geographic localization.

2.2 | Sampling scheme, aboveground biomass estimation, and soil sampling

We adopted a joint (synchronous and geosystematic) sampling scheme for all variables, to guarantee the compatibility of datasets for all investigated components. Vegetation and litter sampling strived to capture the differing scales of plant influence zones, as outlined in Rhoades (1996). We estimated aboveground biomass of large trees (AGB≥10 cm diameter at breast height) in the circular main plot (25 m radius, 1963 m^2), and the minor vegetation and litter in five subplots (25 and 1 m^2 for minor vegetation and litter, respectively). We obtained topsoil (0–20 cm) as composite samples from the centers of the five subplots. We adapted our sampling scheme in CPA to the different forest structure (regularly spaced tree plantation) contrasting with all other systems. Instead of a circle, we used three quadrangular main plots of 25 × 25 m. The subplots and transects were sampled as above. Further details about the sampling scheme are presented in previous studies (Cardozo et al., 2015; Leite et al., 2016) and can be found in Figure S1.

Large biomass components were estimated allometrically via diameter-based equations for mature rainforest trees (Overman et al., 1994), secondary forest trees (Nelson et al., 1999), lianas (Gehring et al., 2004) and, when present, babassu palms (Gehring et al., 2011), and also via conversions between the dbh and the diameter measured at a 30-cm height for smaller vegetation components (Gehring et al., 2008). The following were distinguished: large vegetation (trees with dbh ≥10 cm and palms >2 m high) (AGB≥10 cm dbh); mid-sized vegetation (trees, shrubs, and lianas with dbh <10 cm, and palms <2 m high); and small vegetation (herbaceous and shrubs <1.30 m high). Small vegetation was estimated destructively and jointly with the litter layer. For statistical analyses, mid-sized and small vegetation were combined (AGB < 10 cm dbh). The biomass of fallen logs in transects (Brown, 1997; Chave et al., 2005; Van Wagner, 1968) and standing dead logs in the circular main plots were quantified following the line-intercept method described in Arevalo et al. (2002). We estimated small (<1 m height) vegetation and the litter layer (distinguishing between leaves and twigs) destructively, dry matter contents were determined after oven-drying at 65°C until constant weight.

We sampled 0–20 cm soil in each sub-quadrant as specified in Figure S1 resulting in five samples per site for the spontaneous forests, EFA, and HA, and six samples for the CPA sites. Soil biological samples were stored on-field at 4°C, and subsequently frozen at −80°C for DNA extraction. All sampling was performed during the rainy season (from mid-January to early April 2015).

As indicators of topsoil physical quality we determined soil bulk density (volumetric rings) and soil texture (via a pipette method), following procedures described in Klute et al. (1986). For topsoil chemistry, we followed the routines of the Agronomic Institute of Campinas-IAC (van Raij et al., 2001) measuring the following indicators: pH, determined via soil suspension in 0.01 M $CaCl_2$; soil organic matter, determined by the Walkley-Black digestion method; plant-available P, estimated via extraction with a synthetic anion exchange resin Amberlite IRA-400; exchangeable K, determined via Mehlich I extraction; Ca and Mg, determined via KCl extraction; and H + Al, determined by the Shoemaker–McLean–Pratt (SMP) method.

2.3 | Amplicon-based 16S and 18S rRNA gene analyses

Total soil DNA was extracted from 0.25 g of soil using the Power Soil kit (Mobio), following the manufacturer's instructions. To assess the impact of treatments on the bacterial and fungal communities, we sequenced the 16S and 18S rRNA genes. The 16S rRNA sequencing target the V4 to amplify the archaeal/bacterial communities using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011). For the 18S rRNA gene, marker selected target the fungal community via using the primers FR1 (5′-AICCATTCAATCGGTAIT-3′) and FF390.1 (5′-CGWTAACGAACGAGACCT-3′) (Verbruggen et al., 2012) which contains a small modification to detect *Glomeraceae*. The sequences were PCR amplified using barcoded primers (Caporaso et al., 2012).

The 16S rRNA gene amplification for library preparation were performed using the C1000 thermocycler (Biorad) with the following thermal conditions: 95°C for 5 min; 35 cycles of 95°C for 30 s, 53°C for 30 s, and 72°C for 60 s; and 72°C for 10 min. A 25-μl reaction contained 2.5 μl of 10× PCR buffer, 2.5 μl of dNTPs (200 μM), 0.25 μl of each primer (0.1 pmol/μl), 0.2 μl of FastStart Exp polymerase (0.056 U), and 1 μl of DNA (0.6 ng). The 18S rRNA gene amplification reactions were performed using 5 micromolar of each primer, 2 mM dNTPs (Invitrogen), 0.5 μl of BSA, 10 PCR buffer, 0.56 units of Fast Start Exp-Polymerase, and 1 microliter of sample DNA template in a total reaction volume of 25 μl. The PCR was conducted with initial incubation of 5 min at 95°C followed by 25 cycles of 30 s at 95°C, 1 min. at the annealing temperature of 57°C, 1 min. at the extension temperature of 72°C, followed by a final extension for 10 min at 72°C. The reactions were performed in triplicate and a negative control was included. The amplicon sizes were checked by gel electrophoresis. PCR products were purified using the Agencourt AMPURE XP system (Beckman Coulter) to remove primer dimers, quantified using Fragment Analyzer (Perkin-Elmer Corp.), and mixed in equimolar amounts for sequencing using Illumina MiSeq (Illumina Inc.).

Sequences of the 16S and 18S rRNA partial gene amplicons were processed using dada2 workflow (Callahan et al., 2016) on a 32-node server running Linux Ubuntu 14.4. The forward and reverse primer sequences were removed from the FASTQ file of each sample using Flexbar version 2.5 (Dodt et al., 2012). Reads were filtered based on sequence quality by running the Sickle tool (minimum quality score of 25 and minimum length of 150) (Joshi & Fass, 2011). Taxonomic information for each ASV was added to the BIOM file using the SILVA rRNA gene database (version 132) (Quast et al., 2013). Both bacterial and fungal communities were accurately characterized at genus level. The sequences were deposited in ENA database. In total, the sequencing resulted in 3,308,164 reads for bacteria and 3,334,355 for fungi, with an average of 13,726.82 reads of bacteria and 13,835.5 reads of fungi per sample. The rarefaction curves for both bacterial and fungal communities are presented in Figures S2 and S3.

2.4 | Statistical analysis

Analysis of the soil microbial community using next-generation sequencing data is challenging. Several studies pointed out the potential bias associated with the method of DNA extraction (Dimitrov et al., 2017), PCR, and sequencing (Kennedy et al., 2014). Altogether, these potential problems might mislead interpretations, especially when they are combined with distance measures traditionally adopted for the investigation of clustering and similarities between treatments (Warton et al., 2012). The composition of microbial communities in soil is tightly connected with soil characteristics (Cassman et al., 2016), nutrient availability (Delgado-Baquerizo et al., 2017; Pan et al., 2014), plant biomass (Aponte et al., 2013), and symbiotic interactions (Albornoz et al., 2022). These parameters are in turn connected with land use and management practices (Barnes et al., 2014). These relationships make the use of environmental variables as predictors of the microbiome prone to bias toward collinearity and overfitting (Dormann et al., 2013). To circumvent this problem, we adopted the generalized joint attribute modeling (GJAM) (Clark et al., 2017). This model allows one to include variables of different types and to analyze them jointly, thus revealing the regression coefficients of the effects of different land uses in the relative abundance of taxa within the soil microbiome, as constrained by the compositionality (Gloor et al., 2017), the aboveground biomass and soil factors. For the microbial community data, GJAM also allows us to evaluate the model fit for both the abundance (in our case, the relative abundance, constrained by the compositionality) and the diversity (given by the Shannon index). According to those preliminary analysis the model we obtained a good explanatory capacity for the changes in microbial relative abundance (Figure S4a) though we underestimated the richness and overestimated the Shannon diversity (Figure S4b,c). Based on this outcome, we focused our analysis on shifts of the microbial community at the genus level, for which we obtained the best fit for understanding community variability. Since GJAM is based on Bayesian statistics, we obtained regression coefficients and considered them as significant when the 95% of the highest posterior density (HPD) interval does not include zero. In our study, zero represents the null hypothesis that there are no differences between the land-use systems (secondary successional stages, agroforests, and MFs). For the current study we focused on

the significant regression coefficients as a proxy of the changes in aboveground–belowground components (plant biomass, soil factors, and microbial communities) of the different land uses. Subsequently, we performed a hierarchical clustering analysis (Euclidean distance and Ward algorithm) of the different regression coefficient to identify similarities in the responses to the land use. Lack of occurrence of MFs in every county rendered geographic distance as a potential factor affecting the results and was, therefore, included in modeling. GJAM allows to include random effects, which accounts for the within site replicates and regional (between site clusters) variability. Another advantage of the GJAM approach is the possibility to perform conditional prediction that allowed us to simulate scenarios for some specific set of dependent variables. We adopted this tool to simulate a scenario where all the land-use systems (agroforests and secondary regrowth) have the same microbiome found in the MFs (microbiome as predicted by the model). The intention here was to compare how much the soil factors and plant biomass should differ from the original value recorded during the measurements in each sampling site to achieve the microbiome of the mature rainforest. The level of change for each variable was summarized as a ratio of change (the ratio between the simulated value and the original value found in each site). We employed this approach to model the effects of environmental factors (the AGB and soil factors) on the community structure and interactions. All the above-mentioned analyses of bacterial and fungal community were done at genus level. All analyses were performed in R using a combination of the packages gjam (Clark et al., 2017), pvclust (Suzuki & Shimodaira, 2006), ggplot2 (Wilkinson, 2005), and flipPlots (Displayr, 2021).

3 | RESULTS

3.1 | AFS are bacteria driven whereas secondary succession is fungal-driven ecosystems

We use mature rainforest (MF) without any visible human perturbation as a tropical rainforest standard and compare these with three different agroforestry practices and with spontaneous secondary successions following slash-and-burn agriculture. The secondary successions (YSF, MSF, and OSF) had the most similar characteristics across sites (Figure 2a,b). The aboveground regrowth is characterized by an increasing number of *Ascomycota* and *Basidiomycota* fungi (Figure 2a, clusters C1–C3) that clustered with the MF (Figure 2b). A second cluster grouped the homegardens (HA) together with the commercial plantation agroforests (CPA), and the enriched agroforests (EFAs), likely due to reduced proportion of some specific fungal

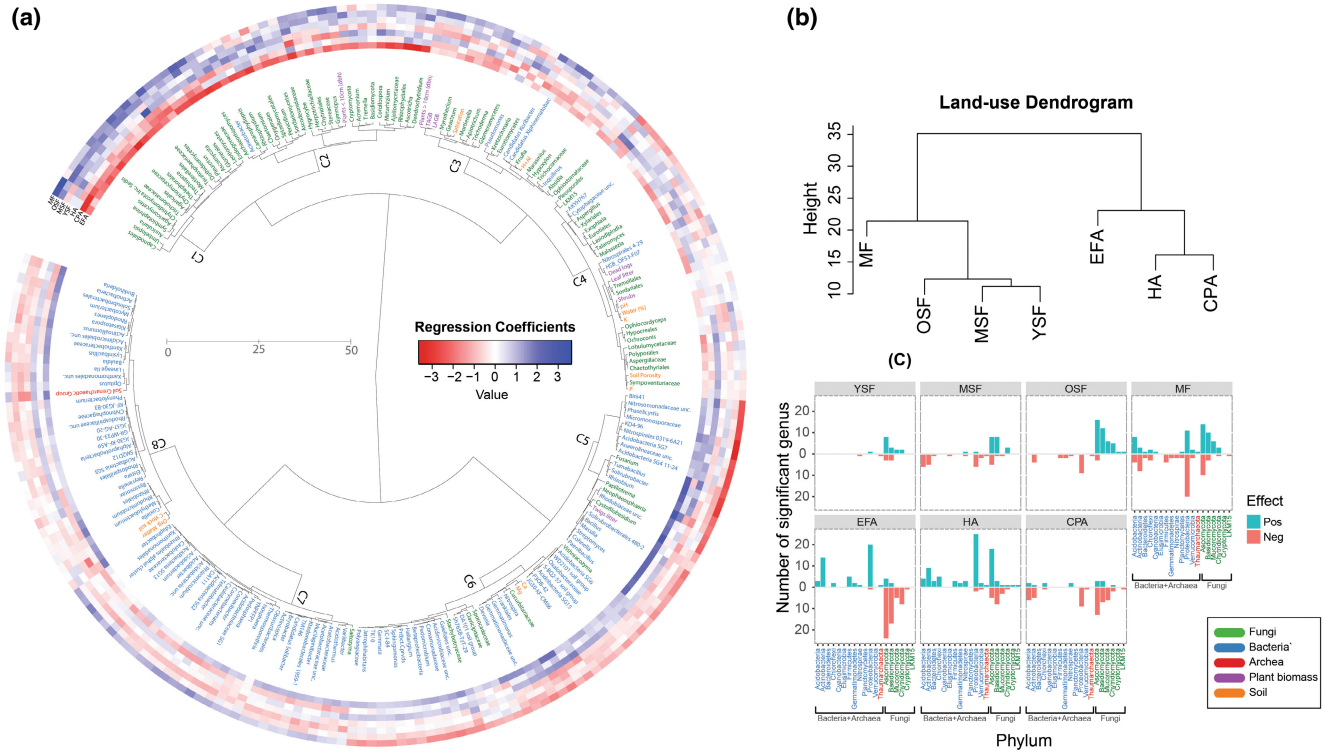

FIGURE 2 Agroforestry systems divergence and secondary succession convergence toward the aboveground–belowground interactions of mature rainforests. (a) Regression coefficient showing the shifts in the soil microbiome, plant biomass, and soil factors, (b) the similarities between land-use systems (Euclidean distance and Ward algorithm), (c) number of genera that exhibited positive (blue) or negative (red) significant regression coefficients within phyla of bacteria, archaea, and fungi in different land use systems, MF, mature forest (>100-year-old, 12 sites); OSF, old secondary forest (>30-year-old, seven sites); MSF, mid-age secondary forest (15–20-year-old, six sites); YSF, young secondary forest (5–12-year-old, five sites); EFA, enriched fallow agroforestry (six sites); HA, homegarden agroforestry (13 sites); CPA, commercial plantation agroforestry (seven sites).

genera of *Ascomycota* and *Basidiomycota* phyla (Figure 2a, clusters C1–C4) and bacterial genera of *Proteobacteria* phylum (Figure 2a, clusters C5–C6), with marked differences from MF (Figure 2a–c).

Agroforest soils had higher topsoil pH, Ca-, and Mg availability, lower soil porosity, soil saturation, and soil moisture than the spontaneous forests. CPA and HA also had higher concentration of available P and soil organic matter than the YSF and MSF spontaneous forests. We observed significant increase in soil organic matter along the spontaneous succession (YSF, MSF, and OSF). Aluminum saturation (H+Al) also increased along secondary succession being highest in the MF sites.

The MF sites showed more similarities with secondary forests than with AFS (Figure 2b). In general, all spontaneous forests exhibited a high proportion of different fungal genera of the phyla *Ascomycota* (YSF = 8, MSF = 8, and OSF = 16), *Basidiomycota* (YSF = 3, MSF = 6, and OSF = 9) (Figure 2c), and *Mucoromycota* (YSF = 2 and OSF = 6). Within the *Mucoromycota* phylum, we found two unclassified genera of arbuscular mycorrhizal fungi (AMF) (uncultured *Glomeromycetes* and *Glomerales*), both significantly more abundant in MF and in secondary succession forests (OSF and YSF) than in the AFS. In contrast, the AFS had a higher relative abundance of numerous bacterial genera (EFA = 44, CPA = 7, and HA = 53), yet only few fungal taxa (EFA = 6, CPA = 7, and HA = 25). The bacteria belonged to the phyla *Acidobacteria*, *Actinobacteria*, *Bacteriodetes*, *Cloroflexi*, *Chytridiomycota*, *Planctomycetes*, *Proteobacteria*, *Thaumarchaeota*, and *Verrucomicrobia*. Interestingly, *Glomeromycetes* clustered with variables of aboveground biomass (C3) but not with available P-content in soil (C4). *Glomeromycetes* is a class of fungi that comprise AMG.

The collective changes in plant biomass and soil factors also contributed to the distinction between the land-use systems (Figures 2a and 3). Overall, the MF systems had the highest values for total aboveground biomass (TAGB), followed by the OSF and HA. Moreover, the regression coefficient of aboveground biomass shifts from negative to positive from the YSF to the OSF, suggesting a gain of plant biomass along the spontaneous secondary regrowth (Figures 2a and 3). The increase in the regression coefficient of TAGB from YSF to OSF and its similarity with the MF reflect the regrowth of plant biomass from secondary forests to mature rainforest level. Considering only AGB and soil factors, OSF (>30 years) even clustered together with MF. Our results show that the soil microbiome along secondary succession also seemed to recover more in the direction of the MF microbiome. By contrast, the AFS (EFA, CPA, and HA) clustered together and differed markedly from the secondary forest successional trajectory. This clustering of AFS distant from spontaneous was driven by an increased importance of bacterial communities, followed by changes in the understory biomass (plants <10 cm diameter at breast height—dbh) and in soil nutrients, mainly high K availability, and a tendency toward low Mg availability.

Figure 3 summarizes the general trends in microbial community shifts, changes in plant biomass and soil factors, as a departure from our null hypothesis (no differences between the land-use systems, Section 2.4). The spontaneous forests became increasingly different along the natural succession. The number of significant positive shifts (*pos) increased from 16 in the YSF to 28 in MSF, and 48 in OSF sites. However, they remained distinct from the MF that presented 71 significant positive changes. Similar patterns appeared for the negative shifts (*neg), for which the YSF started with 17 significant changes, followed by 34 in the MSF, 25 in OSF, and 70 in MF sites. Apart from that, the gray curves indicated whether the same set of positive and negative coefficients remained significant or not from

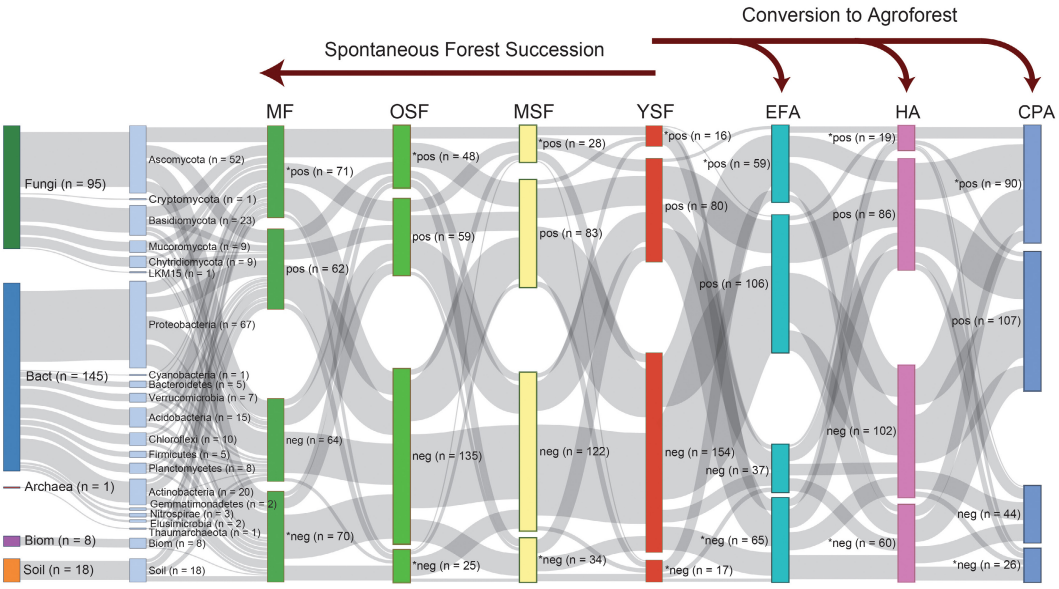

FIGURE 3 Sankey diagram that represents the changes in MF, mature forest (>100-year-old, 12 sites); OSF, old secondary forest (>30-year-old, seven sites); MSF, mid-age secondary forest (15–20-year-old, six sites); YSF, young secondary forest (5–12-year-old, five sites); EFA, enriched fallow agroforestry (six sites); HA, homegarden agroforestry (13 sites); CPA, commercial plantation agroforestry (seven sites).

one land use to the other. From that, the spontaneous forests (YSF, MSF, and OSF) increased similarly to the MF as the natural succession progress. On the other hand, the different agroforest systems follow a distinct path that is represented by the oscillating curves, an outcome of the increased importance of bacterial communities in those land-use systems.

All three AFS fostered bacterial groups with clear differences between them. Homegarden agroforestry promoted the abundance of bacterial groups in clusters C5, C6, and C8. Within these clusters, the top five strongest shifts in bacterial abundances that characterize the HA were: uncultured bacterium from the BIrii41 family (C5), *Solirubrobacter* (C5), *Nitrospira* (C6), *Rhizobium* (C5), and *Frankiales* (C6). Only few groups of fungi more abundant in the HA were also common in the MFs: six fungal taxa from cluster C2 (*Corollospora*, *Metarhizium*, uncultured *Ajellomycetaceae*, *Ascotrichia*, unc. *Rhizophydiales*, and *Dendrochytridium*); three fungal taxa from cluster C3 (*Myrothecium*, *Mortirella*, and *Apiotrichum*); and six uncultured fungi from cluster C4 (*Tremellales*, *Sordariales*, *Lobulomycetaceae*, *Polyporales*, *Aspergilaceae*, and *Chaetothyriales*). In CPA sites, only eight fungal genera were also common in the MF system: *Archeorhizomycetes*, *Hygrocybe*, unc. *Stereaceae*, unc. *Rhizophydiales*, *Ascotricha*, and *Dendrochytridium* from cluster C2; and unc. *Tremellales* and unc. *Sordariales* from cluster C4. The other groups that characterize the CPA land use system are mainly composed by bacterial groups notably the top 5: *Chloroflexi* KD4-96, *Acidobacteria* Subgroup 7, *Nitrospirales* 0319-6A21, *Nitrospirales* 4-29, and unc. *Frankiales*. Finally, EFAs were—according to the dendrogram in Figure 2b—the agroforestry system closest with the MF. This is likely because the both systems promoted the abundance of the same taxa present in cluster C7 (22 taxa in total, with only one fungal genus, *Saitozyma*). The only archaea taxon that significantly responded to the different land-use systems (unc. Soil Crenarchaeotic Group) was likewise abundant in both EFA and MF sites. The top 5 most dominant group of bacteria that characterize the EFA system are: *Tumebacillus*, *Solirubrobacter*, *Massilia*, *Bacillus*, and unc. *Actinobacteria* 480-2, all of them from cluster C5.

Our model also revealed which microbial genera, plant biomass, and soil factors responded similarly to the land-use changes via the hierarchical clustering of variables (Figure 2a, Supplementary Results present a more detailed description of each cluster). Cluster C1 grouped the response of 19 different fungal genera that belonged to eight different classes (seven *Agaricomycetes*, three *Chytridiomycota incertae sedis*, three *Dothideomycetes*, two *Mucoromycota incertae sedis*, one *Glomeromycetes*, one *Leotiomycetes*, one *Xylonomycetes*, and one unclassified genus from the phyla *Ascomycota*), the majority of them increased their relative abundance along secondary forest succession (YSF → MSF → OSF). Cluster C2 was also composed largely by fungal genera (24 in total), grouped in nine distinct classes (six *Sordariomycetes*, five *Chytridiomycota incertae sedis*, four *Agaricomycetes*, four *Eurotiomycetes*, one *Archaeorhizomycetes*, one *Pezizomycetes*, one *Tremellomycetes*, and two unclassified genera belonging to the phyla *Basidiomycota* and *Cryptomycota*, respectively), as well as one *Gammaproteobacteria* of the genus *Acinetobacter*. Cluster C2 also contained the regression coefficients for the changes in understory plant biomass (Plant<10 cm dbh), suggesting that those fungal genera are associated with the increased gain of understory plant biomass that occurred in spontaneous forests, but also related with the reduced importance of this biomass in AFS.

From Cluster C3 onwards, we observed a mixture of variable groups. This cluster contained four bacteria genera (*Pseudomonas*, Candidatus *Koribacter*, Candidatus *Xiphinematobacter*, and *Inquilinus*), and 14 different genera of fungi from six different classes (five *Sordariomycetes*, three *Eurotiomycetes*, two *Agaricomycetes*, two *Mucoromycota incertae sedis*, one *Glomeromycetes*, and one *Tremellomycetes*). In general, the variables in this cluster presented positive coefficients for the spontaneous forests (YSF, MSF, OSF, and MF) and negative shifts for the AFS (EFA, HA, and CPA), with some exceptions. Notably, the fungal genera *Trichoderma*, *Apiotrichum*, *Mortierella*, *Geastrum*, *Myrothecium*, and an unclassified genus from the class *Glomeromycetes* aggregated in cluster C3 with variables of TAGB, living aboveground biomass, and the biomass of plants >10 cm dbh (the group of plants that represent the canopy) (Figure 2a). Those variables shift from negative to positive coefficients along secondary succession but are also positive in the HAs.

In cluster C4, we found 19 genera of fungi associated with shrub aboveground biomass and variables of leaf litter and dead logs, all the fungi general belonged to six different classes (six *Eurotiomycetes*, four *Dothideomycetes*, four *Sordariomycetes*, one *Agaricomycetes*, one *Chytridiomycota incertae sedis*, one *Tremellomycetes*, and one unclassified genus from the phyla LKM15). Cluster C4 also comprised the shifts of five soil factors (pH, soil water content, soil porosity, K, and P) and four different taxa of bacteria (AKYH767, unclassified *Cytophagaceae*, unclassified *Nitrospirales* 4-29, and HSB OF53-F07).

For clusters C5 and C6, variables more relevant in the AFS predominate. Cluster C5 contained 19 bacterial genera grouped in 11 classes, namely: four genera of *Bacilli*; *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, and *Thermoleophilia* with two genera each; and *Acidobacteria*, *Anaerolineae*, *Holophagae*, KD4-96, and *Nitrospira* with one genus each. Cluster 6 comprised 27 genera of bacteria from 15 distinct classes: the *Betaproteobacteria*, *Deltaproteobacteria*, and *Alphaproteobacteria*, with five, four, and three genera, respectively; the *Acidobacteria*, *Actinobacteria*, and *Gemmatimonadetes* with two genera each; and *Acidimicrobiia*, JG30-KF-CM66, *Nitrospira*, *Phycisphaerae*, *Planctomycetacia*, S-BQ2-57 soil group, *Spartobacteria*, *Sphingobacteriia*, *Thermoleophilia*, and TK10 with one genus each. All those microbes are significantly more abundant in the AFS than in the spontaneous forests.

Clusters C7 grouped 31 bacteria genera from 10 classes that become increasingly relevant along secondary succession but were also important in the EFA system: *Acidobacteria* (9), *Alphaproteobacteria* (8), *Actinobacteria* (3), *Thermoleophilia* (3), *Gammaproteobacteria* (2), *Ktedonobacteria* (2), *Melainabacteria* (1), OPB35 soil group (1), *Planctomycetacia* (1), and *Sphingobacteriia*

(1). Finally, cluster C8 represents the bacteria and the archaea that became more relevant (significant positive coefficients) in the EFA and HA systems, with the only exception of five bacterial genera (*Coxiella*, *Methylobacterium*, *Rhodomicrobium*, unclassified *Rhizobiales*, and *Byssovorax*) which were abundant only in the EFA systems.

Altogether, bacterial community responses generally tracked trends found in the soil factors (Ca and Mg in cluster C6, and soil organic matter in cluster C8) and only 10 bacterial genera were related with changes in twigs biomass (cluster C5). In summary, bacterial community played a major role in the microbiome of the three AFS whereas soil fungal community increased in relative abundance along secondary succession, (Figures 2c and 3).

3.2 | Conditional modeling provides guidelines for agroforestry systems to better mimic the mature forest

The joint analysis of the microbiome, the aboveground biomass, and soil physical and chemical characteristics allowed us to simulate scenarios which evaluate how vegetation biomass and soil factors would need to shift under a specific condition (Section 2). Since our goal was to investigate the capacity of AFS to mimic the aboveground–belowground interactions found in mature rainforests, we simulated a scenario in which all the different land use systems have the microbial community estimated for the MFs, thus allowing the model to obtain the necessary values of both plant and soil factors required to achieve that condition. This analysis allowed us to identify the site-specific variables that would need to change in order to attain the MF microbiome.

The results from these simulations returned the ratio of changes from each system (Figure 4). The secondary forests (OSF, MSF, and YSF) showed the lowest ratios of change. For the OSF, to have the same microbiome of MF, the biggest relative changes would need to occur within the biomass of shrubs with a median increase of 2.3 times the original value, followed by a 1.8-fold increase in the biomass of plants >10 cm dbh. For the MSF, the microbiome would require more than fourfold increase in the shrubs and dead logs. Finally, for the YSF, the microbiome would require 5.3-fold increase in shrubs biomass, 4.4-fold increase in dead logs, and a 4.2-fold increase in plants >10 cm dbh. Only a small percentage of the secondary forest sites required ratios of changes above 2.5-fold, 25% of YSF sites for the available P and 36% of YSF for available K, in 25% of the MSF sites available K-content would need to increase by 5.2 times, also in one OSF site available K would need to increase by 5.1 times.

In marked contrast, all three AFS would require very large ratios of change, especially for the plant biomass variables. Our simulation results show that, in order to achieve the MF microbiome, EFA would require 3.7 times more dead logs biomass, as well as 2.6 times more litter mass and shrub biomass. The CPA systems would require similar increases in the dead logs and shrub components (more than 3.2 times) and 2.3 times increase in plants <10 cm dbh. By contrast, the soil factors were less relevant than vegetation parameters along the spontaneous secondary forest succession where most of the median values were close to the ratio of 1. Of the three AFS, the HA exhibited the highest ratios of necessary changes for the soil variables, requiring an increase in the soil nutrients (P, K, Ca, and Mg) for more than 75% of their sites (Figure 4). Despite that, for the other AFS (EFA and CPA) the ratios of changes were below twofold for 75% of their sites. Notably, some of the sites should even reduce the availability of soil nutrients such as the soil P content in EFA systems and nearly half of the CPA sites. In summary, the changes in aboveground biomass variables played a major role in allowing the different AFS to reach the microbiome of Amazon MF.

4 | DISCUSSION

4.1 | Agroforestry system divergence and spontaneous forest convergence toward the mature forest microbiome

We obtained an ecosystem perspective of the effects of agroforestry practices and of secondary forest succession by combining aboveground (plant biomass) with the belowground (soil factors and microbial community composition) components in a generalized joint species attribute model (Clark et al., 2017).

Mature rainforests (MFs) are characterized by high biomass of living aboveground plants (large vegetation and shrubs), but also dead logs, low pH values, high aluminum saturation (H + Al), soil water content, and K-content. The soil microbiome was mainly composed by fungal groups, but with some bacterial taxa with relevance, as indicated by the cluster C7. Fungal community also played a major role in forming the soil microbiome of all spontaneous secondary forests (YSF, MSF, and OSF). We also observed a secondary succession shifting the coefficients of the secondary forests toward more similarity with the MF. For example, the variable aboveground biomass for the larger plants (bigger than 10 cm dbh, cluster C3) started with a negative coefficient in YSF and MSF but became positive in the OSF system. The aboveground biomass of smaller plants (less than 10 cm dbh, cluster C2) shows similar trends with regression coefficients that become strongly positive from YSF to OSF. Aboveground biomass accumulation along secondary forest succession following shifting cultivation land use has been described in other studies (Jakovac et al., 2016; Pollini, 2014). In summary, spontaneous secondary forests become more similar to the mature rainforest ecosystem, but fully recovering the soil microbiome will require longer periods of fallow.

On the other hand, the three AFS followed a diverging path when compared with the spontaneous secondary succession. The agroecosystem profile of EFAs, HAs, and CPAs clustered together due to their capacity to promote higher abundance of bacterial communities. The main elements of changes toward a more bacterial-driven agroecosystem are likely the loss of mid-sized vegetation

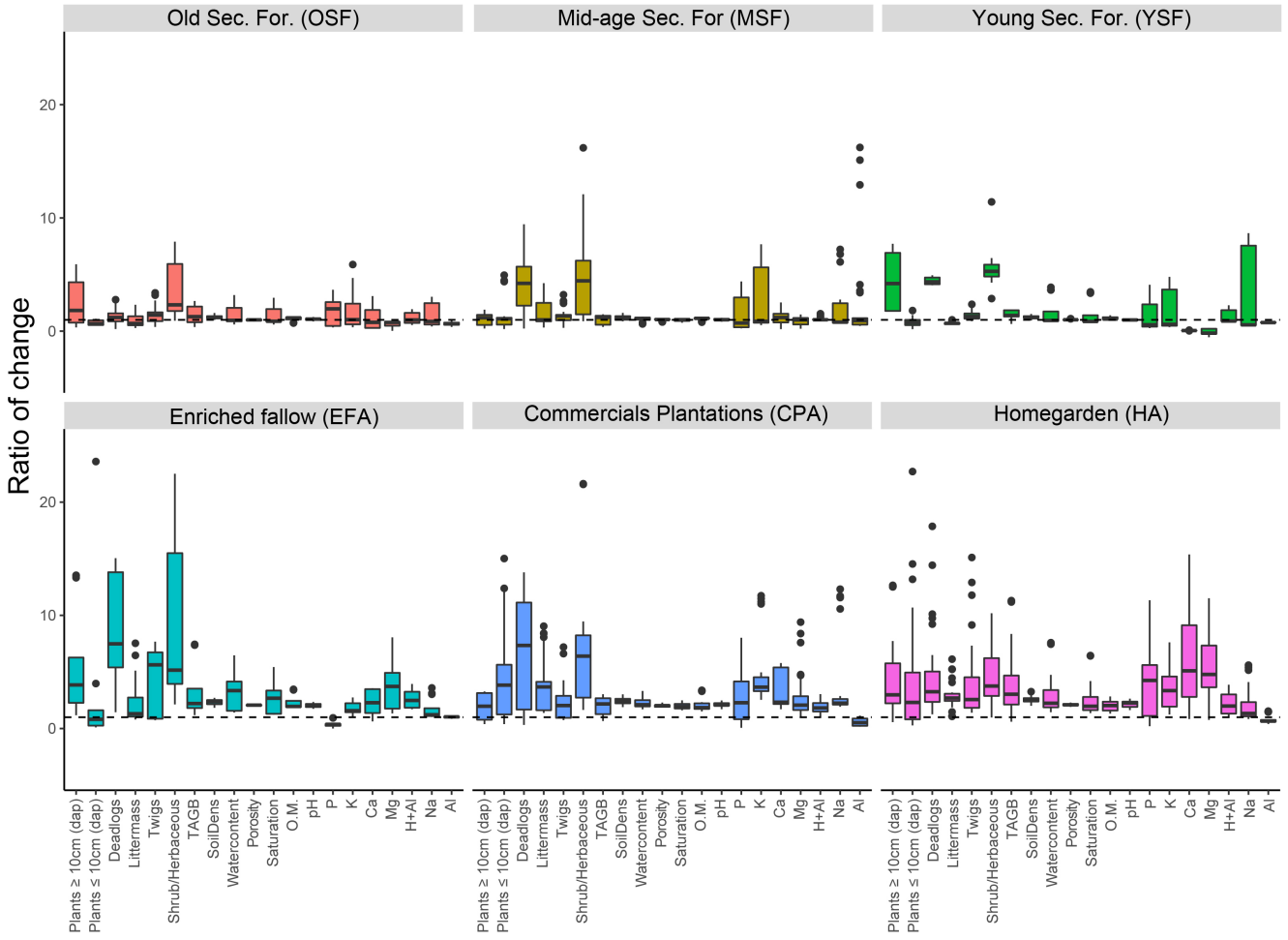

FIGURE 4 Ratio of changes necessary for all land-use systems to attain the mature rainforest microbiome. The closer the value is to one (horizontal dashed line in the graph) the closer the system already resembles the microbiome estimated for the mature rainforest (MF) standard. OSF, old secondary forest (>30-year-old, seven sites); MSF, mid-age secondary forest (15–20-year-old, six sites); YSF, young secondary forest (5–12-year-old, five sites); EFA, enriched fallow agroforestry (six sites); HA, homegarden agroforestry (13 sites); CPA, commercial plantation agroforestry (seven sites).

(dbh < 10 cm), increasing pH and soil nutrient contents (P, K, Ca, and Mg). Specific management practices in agroforestry (e.g., pruning, weeding, clearing the understory) explain the reduction of biomass of smaller plants and represent the farmer's need to clear area for planting desired trees and crops and managing their access to sunlight. The nutrient inputs regularly applied in CPA systems, as well as nutrient hotspots caused by sweep-and-burn in homegardens (Leite et al., 2016; Winklerprins, 2009), selected fast-responding bacteria (Alpha-, Beta-, Delta, and Gammaproteobacteria) and likely explains the increased overall abundance of bacteria (Delgado-Baquerizo et al., 2017). EFA are the type of agroforestry system with the clearest intention of benefiting from mimicking the secondary succession while providing food, crops, and wood for the farmers. Interestingly, our results showed that this system differed from all spontaneous forests by promoting the abundance of several bacterial groups in the clusters C5–C8. Mulching caused by slash-and-mulch (chopping and dropping selected plants in the understory) also explains the positive effects in soil organic matter and the promotion of bacterial groups. Altogether, agroforestry practices created new habitat conditions that fostered the microbial community composed mostly by bacteria and archaea, diverging from those in spontaneous forest soils.

Bacteria-dominated clusters (C5–C8) also reflect the impacts of land use on soil Ca and Mg availability, soil organic matter, and soil carbon stocks. On the other hand, clusters dominated by fungi (C1–C4) grouped together with variables of aboveground biomass. The complexity of the soil bacterial community is primarily governed by soil nutrients, and the fungal community is more strongly associated with variables related to plant aboveground biomass. The increased importance of fungi along secondary succession suggests a crucial role of fungal community in the quick recycling of the nutrients. In the tropical rainforests, trees thrive in deeply weathered and nutrient-poor soils by accumulating nutrients in their biomass and efficiently cycling them to avoid nutrient loss via leaching and soil erosion (Cuevas & Medina, 1988). Our findings suggest that fungal communities play a crucial role in nutrient cycling in MF and along secondary forest succession but not in AFS.

A further result of our study is the finding that the changes in *Glomeromycetes* are closely associated with the variables of

aboveground biomass (clustered together in C3), and to a lesser degree related with plant-available topsoil P (present in cluster C4). Arbuscular mycorrhizal fungi are known to strongly affect plant population and community biology and vice versa (Bonfante & Anca, 2009; Tedersoo et al., 2020). Our results suggest a stronger codependence between AMF and aboveground biomass rather than between plant biomass and topsoil P availability. These results are likely the outcome of the vegetation's ability to sustain AMF communities, and the capacity of mycorrhizal fungi to access sources of P in the soil that are less available to the plants (Bolan, 1991; Guo et al., 2016). *Glomerales* were more prevalent in mature rainforests than in the AFS, this difference was most pronounced in plantation agroforestry (CPA) systems. We, therefore, confirm the relative importance of AMF to the secondary succession and their reduced relevance for the AFS.

We also noticed an increased importance of the bacterial taxa associated with the nitrogen cycle (e.g., *Rhizobium*, *Frankiales*, *Nitrospira*, *Nitrospirales* 0319-6A21, and *Nitrospirales* 4-29). This is another characteristic in which the AFS diverge from the secondary successional path. Previous studies indicated that along succession the N cycle becomes less relevant and that OSFs and mature rainforest are more P limited than N limited (Davidson et al., 2004, 2007). The reduced importance of microbes related to the N cycle and the negative regression coefficients for P availability coupled with the increased importance of AMF in the spontaneous secondary forests and mature rainforests suggest the role of N–P trade-off in determining the ecosystem profile for the Amazon rainforests. Brouwer and Riezebos (1998) highlighted that nitrification becomes a key soil process after logging, which likely explains the increased abundance of nitrogen fixers and nitrite-oxidizing bacteria as the top responding bacteria to the agroforestry practices (notably, CPA and HA). Therefore, even a small-scale logging performed in the AFS (e.g., pruning and clearing of the understory) can induce changes in the nutrient cycle and affect the soil microbiome.

The increasing land use pressure throughout the tropics does not allow for strategies relying purely on secondary forest succession, and AFS have been identified as a promising alternative land use (Angelsen & Kaimowitz, 2004; Nair, 2013). Agroforestry systems provide crops, fruits, and wood with a concomitant increase in agroecosystem complexity (Atangana et al., 2014) that mimics the structure of native forests (Young, 2017). The mimicry hypothesis was elaborated by Ewel (1999) and extended by van Noordwijk and Ong (1999), suggesting that AFS are capable of imitating the structure and functions of natural ecosystems, thus benefiting agricultural sustainability. However, our analysis of the soil microbiome reveals that the capacity of AFS to mimic the complex interactions found in mature rainforest is low. As the soil microbiome plays a central role for full maintenance of the ecosystem services sought from forests, AFS should adjust their management practices to strengthen the aboveground–belowground interactions for more sustainable and eco-efficient land-use systems.

4.2 | Key aspects to better mimic the mature forest

With our model-based approach we were able to determine the management strategies for plant biomass and soil factors that would need to be adjusted in order to speed up recovery toward mature rainforest standard. These new agricultural practices are system and site specific but, in general, involve increasing the aboveground biomass (e.g., dead logs, shrubs, and mid-sized vegetation Plants <10 cm dbh for CPA; dead logs, shrubs, twigs, and large vegetation—plants >10 cm dbh for EFA) and/or the soil nutrient availability (e.g., P, K, Ca, and Mg for HA).

However, the bacterial-driven microbiome present in the AFS may be difficult to displace, as this would require that key soil factors and plant biomass double or triple to reach the microbiome as in mature rainforests. Consequently, the goal of mimicking of MF may be unattainable for commonly used agroforestry practices, thus posing a potential obstacle in efforts to restore aboveground–belowground interactions (plant biomass and soil factors) and related functionalities. Most agroforestry practices are considered low-impact land-use practices that maintain similar or even higher aboveground biomass (Cardozo et al., 2022). By modeling the soil microbial community jointly with the aboveground biomass and soil factors, we moved beyond the mere identification of the impacts of each land-use system. Our findings reveal that agroforestry practices reduced the interdependence of the soil microbiome from the vegetation. This may be the result of the reduction of plant–soil interactions caused by agroforestry land management (nutrient inputs, pruning, weeding, etc.), which reflects the efforts in regulating ecosystem productivity toward consumption or market-related production. Manzoni et al. (2012) showed that chemically too homogeneous plant residues do not promote functionally diverse microbial communities. Selection of agroforestry plant species only based on their cash value could cause AFS to exert detrimental effects on the soil microbiome, for instance by reducing the role of fungi (relative to bacteria) in linking above- and belowground ecosystem elements. We also acknowledge the importance of plant diversity in contributing to better mimicking the complex interactions in MF, which goes beyond the scope of our study. Future studies need to jointly model the responses of plant and microbial diversity along secondary forest succession and in AFS. Despite that, our multi-faced approach suggests that changes in land use, whether agriculturally manipulated or as spontaneous secondary succession after shifting cultivation agriculture cause consistent alterations in the tripartite plant–soil–microbe interactions.

Agroforestry system remains as an important alternative to slash-and-burn agriculture and previous studies confirmed that they are capable of recovering carbon faster than in spontaneous secondary forests (Cardozo et al., 2022). Apart from that, all AFS we studied resulted in higher income: cost ratio when compared with slash-and-burning agriculture (Cardozo et al., 2015). Homegarden agroforests have the advantage of maintaining high diversity in rural

areas (Mohri et al., 2013) and species-rich AFS promote food sovereignty (Armengot et al., 2022). Enriched fallow agroforests allow farmers to grow crops and food in areas that otherwise would be used for slash-and-burn agriculture. Finally, CPAs developed by Japanese immigrants in eastern Amazon represent a case of success in promoting large-scale and profitable production of agroforests in the Amazon region (Cardozo et al., 2015). Our findings contribute to improve their agroforestry practices and increase their sustainability via better management of the aboveground–belowground interactions.

AUTHOR CONTRIBUTIONS

Márcio Fernandes Alves Leite, Flávio Henrique Reis Moraes, Guillaume Xavier Rousseau, and Christoph Gehring designed the research; Márcio Fernandes Alves Leite, Ernesto Gómez Cardozo, Hulda Rocha e Silva, Ronildson Lima Luz, Guillaume Xavier Rousseau, and Karol Henry Mavisoy Muchavisoy conducted the sampling in field; Márcio Fernandes Alves Leite, Ernesto Gómez Cardozo, Hulda Rocha e Silva, and Karol Henry Mavisoy Muchavisoy performed the estimation of plant biomass and the physico-chemical analysis of the soil; Márcio Fernandes Alves Leite, Binbin Liu, and Eiko Eurya Kuramae performed the molecular laboratory work and analyses; Márcio Fernandes Alves Leite performed the statistical analyses; and Márcio Fernandes Alves Leite, George Kowalchuk, Christoph Gehring, and Eiko Eurya Kuramae wrote the paper. All authors reviewed the manuscript.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

The raw sequences were submitted to the European Nucleotide Archive (ENA) under study accession number PRJEB31645. The plant and soil data are publicly archived under a CC-BY 4.0 license and can be access from: https://doi.org/10.5281/zenodo.7389839. The script for generalized joint attribute modeling can be found at https://github.com/Leitemfa/AFS_Microbiome.

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