



## Metagenomics of soil microbiome uncovers community homogenization in agricultural landscapes in Cerrado

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### ABSTRACT

Deforestation and land use intensification have been affecting the soil microbiota community, decreasing taxonomic and functional diversity of soil Archaea and Bacteria, and thus affecting key ecosystem functions. Here, we assess the influence of landscape structure and soil physico-chemical properties on microbiota community (Archaea and Bacteria) in agricultural landscapes in the Cerrado ecoregion. We used a metagenomics approach to obtain the soil microbiome community composition in 32 agricultural landscapes, and piecewise structural equation models to conjointly analyze the effects of landscape structure and soil on taxonomic, phylogenetic, and functional alpha diversity. We also analyzed the effects of landscape structure and soil properties on community taxonomic and phylogenetic beta diversity, using multiple matrix regression with randomization. We found that the number and shape of natural vegetation (NV) areas positively affected taxonomic, phylogenetic, and functional soil microbiota alpha diversity. Percentage of pasture and shape of NV had a positive influence on phosphorus content. Percentage of savanna and landscape compositional heterogeneity negatively affected soil organic matter content. However, soil properties had only an indirect effect on the microbiota alpha diversity. Taxonomic and phylogenetic beta diversity and their components, nestedness and turnover, were very low between soil sites, and positively related to the amount of NV in the landscape and soil chromium concentration. Our results show that rather than decreasing Archaea and Bacteria species richness, intensive agriculture is modifying the community's structure homogenizing species composition between landscapes, leading to a dominance of groups more adapted to intensive agriculture production systems.

### 1. Introduction

Natural landscapes have been transformed into agricultural landscapes all over the world, with nearly 50 % of the planet's land covered by crops and pasture (Ritchie et al., 2022). The expansion and unsustainable intensification of agriculture have been causing the loss and fragmentation of habitats, and landscape homogenization, leading to a decline in biodiversity and ecosystem functions (Laurance et al., 2014;

Newbold et al., 2015). Deforestation and land use intensification have also been affecting the soil microbiota community, decreasing taxonomic and functional diversity of soil Archaea and Bacteria, and thus affecting key ecosystem functions (Veldkamp et al., 2020; Peng et al., 2024).

Soil microorganisms play different roles in ecosystems, from soil formation to nutrient input, affecting plant recruitment and growth (Schulz et al., 2013). Functional diversity of microbial communities can

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influence ecosystems' stability, productivity, and resilience to stress and disturbance (Torsvik and Øvreås, 2002). For example, agricultural intensification leads to a reduction in microbiome relative abundance (Ohigashi et al., 2021), and taxonomical and functional diversity (Muñoz-Arenas et al., 2020; Zhou et al., 2021), affecting key specialized functions such as organic matter decomposition (Singh et al., 2014), and reduction of heavy metal soil contamination (Wu et al., 2006a; Kudo et al., 2013).

Vegetation type and land use can strongly influence the alpha and beta diversity of soil microorganism community (Chu et al., 2011). For instance, the conversion of grasslands to intensive agriculture in England decreases microbiome diversity and changes community composition (beta diversity), increasing the abundance of potentially pathogenic taxa in agricultural fields (French et al., 2017). The conversion of tropical forest in rubber plantation in southwest China changes soil microbial community composition and diversity (Lan et al., 2017). In southeast China (Kumar et al., 2022), land use type and soil physico-chemical properties affect community composition in flowering cabbage fields, where Gammaproteobacteria dominates, and *Brassica* fields, where Bacilli bacteria dominates.

Together with vegetation and land use type, agriculture intensification changes soil physico-chemical properties that play an important role in microbial community composition and diversity (Li et al., 2024). Agriculture intensification can increase soil nutrients and heavy metal concentration, due to the high input of agrochemicals and fertilizers, changing microbial community (Meena et al., 2020; Slimane and El-hafid, 2021). However, despite the several studies analyzing the responses of microbiome community to vegetation type and agricultural conversion, most were performed at local scale and site-centered, hindering our understanding of the effects of landscape level changes on microbiome community.

The Cerrado ecoregion, the most species-rich savanna in the world (Colli et al., 2020), is the most important agribusiness region in Brazil. Intensive agriculture has caused the loss of nearly 50 % of its original area in the last 60 years (Alencar et al., 2020), leading to high extinction risk of several species (Colli et al., 2020). Cerrado ecoregion is comprised by a mosaic of different vegetation types, ranging from grasslands, savannas, and forested savannas, to forests such as seasonally dry and riparian forests (Felfili et al., 2004). Soil physico-chemical characteristic is the main local environmental factor determining savanna types and forest boundaries (Furley and Ratter, 1988; Oliveira-Filho and Ratter, 2002). The high heterogeneity in soil and vegetation types in the Cerrado ecoregion results in a diverse microbiome composition, and thus high beta diversity. In these areas, there is a dominance of the Bacteria phylum Proteobacteria, Acidobacteria and Actinobacteria, and Archaea Euryarchaeota, Crenarchaeota, and Thaumarchaeota (Procópio and Barreto, 2021). Vegetation types differ in microbial species richness in Cerrado. For instance, grasslands had lower microbial richness than savannas and riparian forests, which had the highest richness (Catão et al., 2014). Additionally, the replacement of natural vegetation by agroecosystems decreases microbial species richness and change community composition. In savanna, Proteobacteria was the most abundant phylum, while Actinobacteria was the most abundant in pasture, and species richness was higher in savanna than in pasture (Quirino et al., 2009; Silva et al., 2019). Rhizobium, Azospirillum, Xanthomonas, Pseudomonas and Acidobacterium were dominant in savanna, while Rhizobiales and Bradyrhizobium dominated soybean crop field (Souza et al., 2016). Although these studies have addressed the diversity of soil microbiota in Cerrado, largely among different vegetation types or agroecosystems, no study so far analyzed the effects of landscape structure and the interactions with soil characteristics determining microbial community diversity. With the high speed of Cerrado natural vegetation loss due to agriculture expansion and intensification (Alencar et al., 2020), understanding how landscape changes affect the microbiome is of utmost importance for soil conservation and management planning at the landscape level, and to avoid

taxonomic homogenization (Peng et al., 2024).

Here, we fill this gap knowledge addressing the effects of landscape structure and soil physico-chemical characteristics on the soil microbiota community in agricultural landscapes in the Cerrado ecoregion. We used a metagenomics approach to obtain soil microbiome community composition in 32 agricultural landscapes, and piecewise structural equation models (SEM) to conjointly analyze the effects of landscape structure and soil on taxonomic, phylogenetic, and functional alpha diversity. We also analyze the effects of landscape and soil properties on community beta diversity (community dissimilarity among sampling sites), using multiple matrix regression with randomization (MMRR).

Because changes in landscape structure and soil properties affect microbiome community (Li et al., 2024; Peng et al., 2024), we hypothesize that taxonomic (alpha and beta), phylogenetic (beta), and functional (alpha) diversities are affected by agricultural landscape structure and soil physico-chemical properties. We expect that alpha and beta diversity of Archaea and Bacteria are higher in landscapes with (i) higher amounts of natural vegetation (savanna or forest), (ii) higher number of natural vegetation patches, and (iii) higher landscape compositional heterogeneity. However, we expect that alpha and beta diversity are lower in landscapes with (iv) higher amounts of pasture and crops, (v) more edges, (vi) and a higher number of irregular, small, and thin patches of natural vegetation. Intensive farming can increase soil nutrients and heavy metal concentration, changing and homogenizing the microbial community (Meena et al., 2020; Li et al., 2024). Thus, we expect a higher alpha and beta diversity in landscapes with (vi) higher amounts of organic matter in soil, and a lower alpha and beta diversity in landscapes with (vii) a higher concentration of heavy metals in soil.

## 2. Material and methods

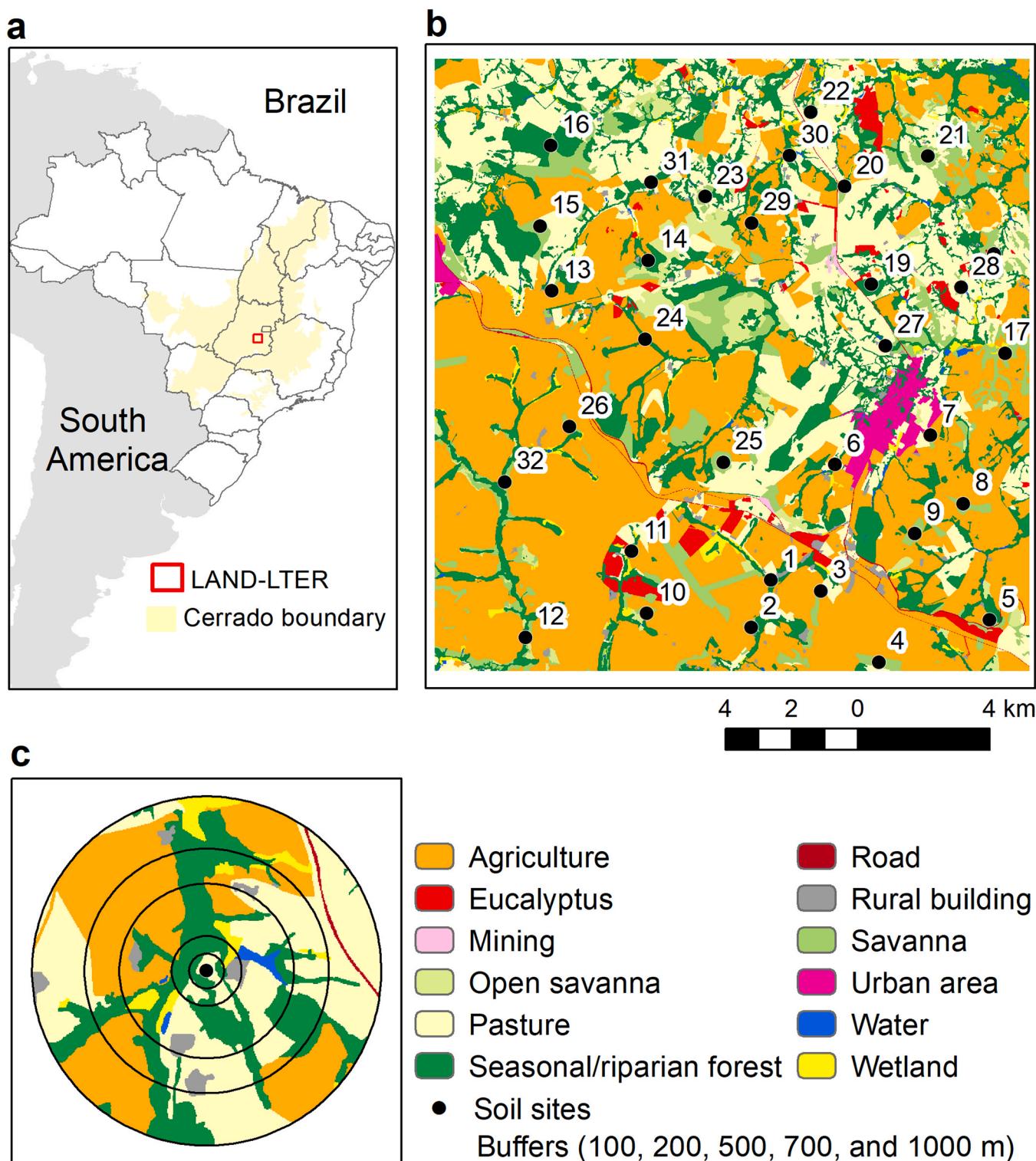
### 2.1. Study area

The study was carried out in an intensive farming landscape within the Long-Term Ecological Research (LTER) experiment LAND-LTER (Agricultural landscape dynamics and impacts on biodiversity), in the Cerrado ecoregion (Fig. 1A). The landscape (Fig. 1B) has 33,400 ha of total area and it is a mosaic of soybean and maize (44.6 %), and pasture (21.6 %), interspersed by small remnants of natural vegetation, such as savannas (9.7 %), riparian and seasonally dry forests (17.8 %), and wetlands (1.1 %) (see details in Santos et al., 2021). We identified the land covers using field inspection and performing a visual interpretation of high-resolution images of Google Earth from 2020 (corresponding to the field work) freely available at the OpenLayer plugin within Geographic Information System Quantum GIS 2.8 (QGIS Development Team, 2017). Later, we made a manual images classification using vectorial tools in QGIS.

The final map had 5 m of spatial resolution, comprising 13 different land cover categories (Fig. 1B): (i) agriculture (maize or soybean); (ii) *Eucalyptus* plantation; (iii) mining; (iv) open savanna; (v) pasture; (vi) riparian forest; (vii) road; (viii) rural building; (ix) savanna; (x) seasonal forest; (xi) urban area; (xii) water; and (xiii) wetland.

### 2.2. Soil sampling design

We collected soil samples in 32 sites in seasonal and riparian forests ( $N = 12$ ) and savannas ( $N = 20$ ). For each site, we collected one soil sampling of 500 g for physico-chemical analyses, and 50 ml of soil in the same area for metagenomics analyses (Fig. 1B, Appendix A Table S1) in January 2020. We collected soil samples at 20–30 cm depth using an alcohol-sterilized auger. Before sampling, we removed the leaf litter from the soil surface. We also manually removed roots, other plant material, and rocks from the samples. For metagenomics analyses, we sieved (2 mm sieve) the soil samples and stored them in Falcon tubes of 50 ml. The soils used for physico-chemical analyses were stored in dry



**Fig. 1.** The Long-Term Ecological Research (LTER) experiment LAND-LTER and the spatial distribution of sampling sites. A) in yellow the location of the landscape in central Brazil in the Cerrado ecoregion which is represented in gray color. B) land cover composition of the study area and the spatial distribution of the 32 soil sampling sites represented in black dots. C) the multiscale approach used to calculate the landscape metrics, considering concentric buffers of 100, 200, 500, 700, and 1000 m of radius size.

plastic bags. All samples were collected on the same day to avoid variation.

### 2.3. Soil physico-chemical analysis

For physico-chemical analysis, we used SOLOCRIA laboratory

facility (Goiânia, BR). We quantified soil organic matter (g/dm<sup>3</sup>), percentage of clay, sand, and silt (g/Kg) and pH (CaCl<sub>2</sub>). For chemical composition, we obtained soil concentrations of heavy metals, which are proxy of high inputs of agrochemicals: cadmium (Cd, ppm), chromium (Cr, ppm), nickel (Ni, ppm), lead (Pb, ppm). In addition, Cd and Cr are present in phosphate fertilizers and can bioaccumulate in soil and crops

(Carvalho et al., 2024). For soil fertility, we measured exchangeable cations calcium ( $\text{Ca}^{2+}$ , cmolc/dm<sup>3</sup>), magnesium ( $\text{Mg}^{2+}$ , cmolc/dm<sup>3</sup>), aluminum ( $\text{Al}^{3+}$ , cmolc/dm<sup>3</sup>), and hydrogen ( $\text{H}^+$ , cmolc/dm<sup>3</sup>), phosphorus (P, ppm), potassium (K, ppm), and zinc (Zn, ppm). We also calculated cation exchange capacity (CEC, meq/100 ml soil) and percent base saturation ( $\text{BS} = [(\text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}+)/\text{CEC}] \times 100$ ). Higher CEC and BS values are associated with more fertile soils.

#### 2.4. Soil metagenomics sequencing and assembly

Soil DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, DK), following the manufacturer's instructions. DNA sequencing was carried out in Novogene Corporation Inc. (Sacramento, CA) facility, using the Nextera XT DNA Library Preparation Kit (Illumina, US) and Illumina NovaSeq 6000 to generate paired-end  $2 \times 150$ -bp reads. All sequence reads were trimmed for adapters using Trimmomatic v. 0.39 (Bolger et al., 2014). Reads were checked before and after filtering with the software FastQC v. 0.11.9 for quality control. With the filtered data, we performed *de novo* shotgun assembly for each sampling site, using metaSPAdes (Nurk et al., 2017) with default parameters (see analysis pipeline in Appendix B Fig S1).

#### 2.5. Taxonomic, phylogenetic, and functional assignment to the sequence reads

To assign the taxonomic profile to the reads to calculate alpha and beta taxonomic diversity, we analyzed the metagenomes using Kraken 2 (Wood and Salzberg, 2014), with RefSeq Archaea and Bacteria database (accessed in April 2023). Then, we estimated taxa abundance for each sampling site with Bracken v. 2.7 (Lu et al., 2017), exclusively accepting taxa with more than 10 assigned sequences. For results visualization, we generated graphs using Pavian v.1.0 (Breitwieser and Salzberg, 2020). The results from all sampling sites were concatenated using the combine\_reports tool implemented in the suite KrakenTools v. 1.2 (<https://github.com/jenniferlu717/KrakenTools>), and data visualization was done with Krona v. 2.8.1 (Ondov et al., 2011). Further, we built the phylogenetic tree using the function KrakenSummary implemented in the package krakenplot (<https://github.com/clintval/krakenplot>).

To calculate functional richness and diversity, we performed sequence functional annotation using the online server Metagenomics Rapid Annotation with Subsystems Technology (MG-RAST) v. 4.0.3 (Meyer et al., 2008), accessed in May 2023, using the reads filtered and free from adapters. We used default settings and removed all possible Eukaryote sequences. For functional assignment, we used the following databases: The Clusters of Orthologous Genes (COGs, Tatusov et al., 1997); the evolutionary genealogy of genes Non-supervised Orthologous Groups eggNOG (Jensen et al., 2007); the KEGG ORTHOLOGY (KO, Kanehisa et al., 2016); and the SEED Subsystems (Overbeek et al., 2014). We retained SEED subsystems annotation for further analysis due to the higher number of sequences annotated.

#### 2.6. Microbial community analysis

For each soil site, we calculated species richness and abundance (the mean number of sequences assigned for each species in each site). We calculated species diversity using Simpson's (S) diversity index with the function *diversity* included in the R package *vegan* v. 2.6–2 (Dixon, 2003). We also estimated Margalef (I) richness index to correct for sampling size, i.e., the total number of sequences generated for each sampling site. Margalef's I is the number of species in the site minus 1 divided by the natural logarithm of the total number of individuals in the sample, here the total number of reads in sequencing for each site. We used the functions *margalef* implemented in the R package *abdiv* v. 2.2.0 (<https://github.com/kylebittinger/abdiv>).

Because functional annotation is based on sampling site reads, and not on species, i.e., packages of functional annotation use the

sequencing reads for comparison with database and annotation, for functional diversity we calculated functional richness as the number of different gene functions identified in each sampling site by the SEED Subsystems annotation, following the functional annotation (see above). We also calculated Simpson's (S) diversity index, classifying each different gene function identified in the site as a different "species" to calculate the index.

For phylogenetic alpha diversity for each site, we calculated the mean phylogenetic distance (MPD) that measures the phylogenetic distance among all species in the site, and mean nearest taxon distance (MNTD) that measures the average phylogenetic distance among the closest taxa in a given assembly (Webb et al., 2002). We used the *picante* v. 1.8.2 R package (Kembel et al., 2010) to calculate the phylogenetic diversity, considering cophenetic phylogenetic tree and weighted abundance.

To analyze dissimilarity in microbiome composition between pairs of sites, we estimated taxonomic and phylogenetic beta diversity. For taxonomic beta diversity we used Sørensen dissimilarity index ( $\beta_{\text{SOR}}$ ) (Bryant et al., 2008), and portioned beta diversity in its components, turnover ( $\beta_{\text{SIM}}$ ) and nestedness ( $\beta_{\text{SNE}}$ ) (Baselga, 2010; Leprieur et al., 2012). Nestedness occurs when the community of sites with smaller numbers of species are subsets of the community at richer sites, reflecting a non-random process of species loss (Baselga, 2010). On the other hand, turnover involves the replacement of some species in different communities by others. To calculate phylogenetic beta diversity, we also used Sørensen dissimilarity index based on the phylogenetic dissimilarity (1-Phylosor index), and its components, turnover and nestedness (Leprieur et al., 2012). Phylosor represents the proportion of shared and exclusive branch lengths among sampling sites, and ranges from 0 (when the sampling sites is identical, and thus shared branch lengths are identical) to near 1 (when the sampling sites comprise distinct species that share no branches in the phylogenetic tree). All analyses were performed using the *betapart* v. 1.5.6 R package (Baselga and Orme, 2012).

#### 2.7. Multi-scale landscape variables

Because the scale of effect (Jackson and Fahrig, 2015) of landscape metrics on the soil microbiota community and soil physico-chemical characteristics are unknown, we calculated nine landscape metrics at five different spatial scales, i.e., generating concentric buffers of different radius size such as 100, 200, 500, 700, and 1000 m (Fig. 1C) around each soil site.

We calculated landscape metrics such as percentage of natural vegetation cover (including all types of natural vegetation), percentage of savanna (savanna + open savanna + wetlands), percentage of forest (seasonally dry + riparian forests), percentage of pasture (%pasture), percentage of agriculture (%agriculture), and dominant matrix type, which corresponded to the matrix with higher proportion in the landscape. We also calculated the average patch shape index (APSI), number of patches of natural vegetation (NP), and edge density. APSI measures the complexity of the patch shape compared to a standard shape (square) of the same size, and increases without limit as the patch shape becomes more irregular with more edge effect (McGarigal et al., 2012). We calculated the shape index considering natural vegetation types as a unique class, and calculated the mean of these values for each landscape, ignoring the 0 values. When the landscape was entirely occupied by a single patch, then shape index = 1.

To account for the effects of the diversity of different land cover classes, we calculated landscape compositional heterogeneity using Shannon Diversity Index (SHDI) (Fahrig et al., 2011). We calculated all landscape metrics in Fragstats (McGarigal et al., 2012).

#### 2.8. Alpha diversity statistical analysis

Because of the complex relationships between landscape structure

and soil physico-chemical characteristics, and their effects on microbiome community, we used piecewise structural equation models (SEM) to analyze both direct and indirect effects of landscape structure and soil physico-chemical properties on microbiome community alpha diversity.

First, we identified the scale of effect of landscape variables as the scale that best explains the variation of the model. We used generalized linear regression (GLM) implemented in the R multifit package (Huais, 2018). The best scale for each variable and landscape metrics (Tables S2 and S3) was selected based on the lowest Akaike Information Criterion. We also analyzed the spatial autocorrelation in response variables using Moran's I test implemented in the *ape* v. 5.6–2 R package (Paradis et al., 2004). We found no significant autocorrelation in any alpha diversity index (Table S4).

Then, to select the most influential variables for inclusion in the SEM analysis, we fitted GLMs to response variables to select the best predictors, with Poisson, Gamma, or Gaussian distributions based on the best distribution fit (Tables S5 and S6). We also verified the multicollinearity among the explanatory variables for their best scale of effect, using the variation inflator factor (VIF, Tables S5 and S6). Models with VIFs < 3 (Zuur et al., 2010) were compared, and the best models were selected based on  $\Delta AIC < 2$ , and AIC weights ( $wAIC > 0.10$ ) (Burnham and Anderson, 2004). We used a 'dredge' model-comparison approach with the MuMin package (Bartoń 2010). We analyzed model residuals using DHARMA package diagnostics (Hartig, 2016), to eliminate models with residual problems (Tables S5 and S6).

Finally, we used the predictors selected with GLMs (Table S6) to build several SEMs, using R version 4.3.2. and packages *lavaan* (Rosseel, 2012), and *PiecewiseSEM* (Lefcheck, 2016). The goodness-of-fit of each SEM was checked using Fisher's C and Chi-squared tests. Models with  $p > 0.05$  indicated a good model fit to the data, thereby providing statistical support for the model, and confirming the absence of missing relationships.

### 2.9. Beta diversity statistical analysis

For taxonomic and phylogenetic beta diversity, and their turnover and nestedness components, we analyzed the relationships with landscape structure and soil variables using Multiple Matrix Regression with Randomization (MMRR) (Wang, 2013) implemented in the *lgrMMRR* function in *PopGenReport* v. 2.2.2 R package (<http://www.popgenreport.org/>). To obtain the matrices of landscape and soil variables, we calculated the difference in landscape metrics at 100 m spatial scale between each soil sampling site and the difference in soil variables. We calculated Pearson's correlation ( $r$ ) between all landscape and soil variables, and excluded those with  $r > 0.5$ , to avoid collinearity between explanatory variables. For landscape, we used %natural vegetation, %agriculture, %forest and dominant matrix. For soil, we used chromium, CEC, %clay, %sand, aluminum, zinc, hydrogen, potassium, and phosphorus. We also used a geographic distance matrix between soil sampling sites to account for spatial autocorrelation. We performed 10,000 random permutations to obtain the statistical significance of matrix regression.

## 3. Results

### 3.1. Community characterization

Whole genome sequencing of the 32 samples generated a total of ~960 million paired reads or ~288 Gbp of sequence data. After trimming and filtering > 946 million paired reads remained in the data set, a mean of > 29 million paired reads per sampling site (Table S7), with high phred score, ranging from 34 to 36.

We found high percentage of novelty and high diversity in the soil microbiome, with ~ 70 % of sequences in each sample classified as unassigned, i.e., from unknown organisms. Sampling sites had an average of 7600 species (Table S8), ranging from 7185 to 8037. Bacteria

dominated in the assigned sequences, accounting for over 99 % of the classified sequences in any sampling site (Table S8).

Most Archaea sequences were assigned to the highly diverse phylum Euryarchaeota, followed by the phylum Thaumarchaeota (syn. Nitrosphaerota) (Fig S2, Table S9). The family Natrialbaceae was the most frequent, followed by Halorubraceae and Haloarculaceae. *Halorubrum* was the most abundant Archaea genus, and *Halobacterium hubeiense* the most abundant species (Fig S2, Table S9).

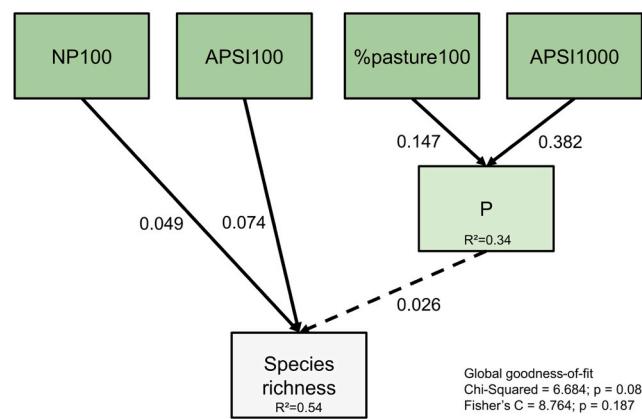
Bacteria sequences were predominantly assigned to the phylum Proteobacteria (syn. Pseudomonadota) in 28 of the 32 sampling sites (Fig S3, Fig S4, Table S10), followed by Actinobacteria (syn. Actinomycetota), the most assigned phylum in four sites. At family level, most Bacteria sequences belonged to Bradyrhizobiaceae and Streptomycetaceae, specifically to five species: *Bradyrhizobium diazoefficiens* (syn. *Bradyrhizobium japonicum*), followed by *Bradyrhizobium erytrophle*, *Burkholderia ambifaria*, *Cupriavidus metallidurans*, and lastly *Delftia acidovorans* (Fig S3, Tables S8 and S10).

We could classify de function of ~ 8.2 million sequences using the 28 categories of the SEED subsystems database (Table S11, Fig S5). Some functional categories such as genes related to iron acquisition and metabolism, phosphorus and potassium metabolism were more abundant in specific sites with dominance of agriculture matrix such as sites 2, 6, 19, 26, 29 and 30 (Fig S5).

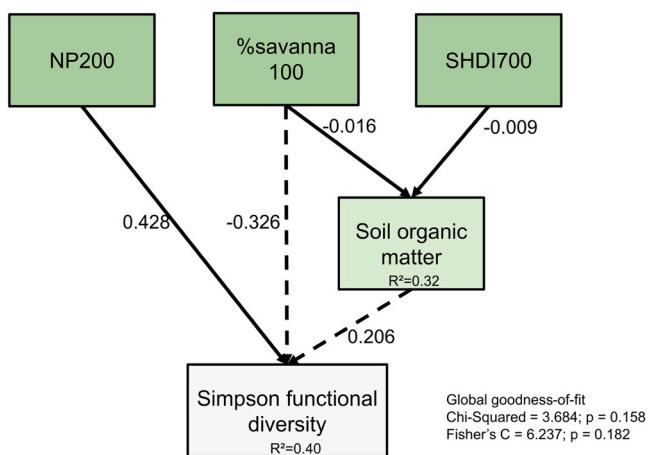
### 3.2. Effects of landscape structure and soil characteristics on alpha diversity

We found that landscape structure affected microbiome diversity and soil properties, but soil had no significant direct effects on microbiome community alpha diversity. Species richness (Fig. 2, Table S12) was positively explained by number of patches (NP, standardize path coefficient  $b = 0.049$ ), and average patch shape index (APSI) both 100 m spatial scale (standardize path coefficient  $b = 0.074$ ). Although soil phosphorus (P) concentration was significantly and positively explained by %pasture at 100 m and APSI at 1000 m spatial scale (Fig. 2, Table S12), its relationship with species richness was not significant. Species richness estimated by Margalef's index was also positively explained by APSI at 100 m spatial scale (Fig S6, Table S12,  $b = 0.008$ ), but again soil P concentration was not significant.

Functional diversity estimated by Simpson's index was positively explained by NP (Fig. 3, Table S12) at 200 m spatial scale ( $b = 0.428$ ). Soil organic matter content was significantly explained by %savanna at



**Fig. 2.** Effects of landscape on soil characteristics, and on microbiome species richness. The final model was obtained following variable selection and model optimization. Bold arrows denote significant relationships ( $p \leq 0.05$ ), and dashed lines non-significant relationships. Numbers next to the arrows are the standardized coefficient values. Models' Structure demonstrates robust performance, as evidenced by Fisher's C and Chi-squared tests ( $p > 0.05$ ), indicating consistency with the data.



**Fig. 3.** Effects of landscape on soil characteristics, and on microbiome functional diversity estimated by Simpson index. The final model was obtained following variable selection and model optimization. Bold arrows denote significant relationships ( $p \leq 0.05$ ), and dashed lines non-significant relationships. Numbers next to the arrows are the standardized coefficient values. Models' Structure demonstrates robust performance, as evidenced by Fisher's C and Chi-squared tests ( $p > 0.05$ ), indicating consistency with the data.

100 m spatial scale and landscape compositional heterogeneity (SHDI) at 700 m spatial scale (Fig. 3, Table S12), but was not significant in explaining functional diversity.

We also found significant models for functional diversity estimated using functional richness (Figs S7, Table S12). However, although functional richness had good fit, it explained lower variance compared to the others (Chi-squared test 3.034  $p = 0.695$ ,  $df = 5$ , Fig S7). For phylogenetic diversity (MPD), we used GLM because we found no significant soil variable predictor (Tables S5 and S6). MPD was best explained by APSI at 100 m spatial scale (standardized  $b = 2.738$ ,  $p < 0.001$ ), and increased with the increase APSI (Fig. 4). For MNTD, no explanatory variable was significant (Table S6).

### 3.3. Effects of landscape structure and soil characteristics on beta diversity

Overall, taxonomic beta diversity and its components (nestedness and turnover) were very low between all soil sites ( $< 0.10$ , Fig. 5A, Table S13). The turnover component ranged from 0.0101 to 0.0510, and was similar to the nestedness component, which ranged from 0.0001 to 0.0666. Beta diversity ( $p = 0.021$ ,  $R^2 = 0.351$ ), nestedness ( $p = 0.001$ ,  $R^2 = 0.288$ ), and turnover ( $p = 0.0004$ ,  $R^2 = 0.124$ ) were related to the

difference in the %natural vegetation in the landscape (Table S14). Both beta diversity and nestedness increased with the increase in the difference of %natural vegetation between soil sites, but turnover decreased (Table S14). Beta diversity was also positively related to the difference in chromium concentration between landscapes (Table S14).

We also found low values of phylogenetic beta diversity (0.0001–0.0893; Fig. 5B, Table S15), and similar values of turnover (0.0116–0.0965) and nestedness (0.0001–0.0889). Phylogenetic beta diversity ( $p = 0.007$ ,  $R^2 = 0.370$ ) and nestedness ( $p = 0.003$ ,  $R^2 = 0.319$ ) were also positively related to the difference in %natural vegetation between landscapes (Table S16). Phylogenetic beta diversity was also positively related to the difference in chromium concentration (Table S16).

## 4. Discussion

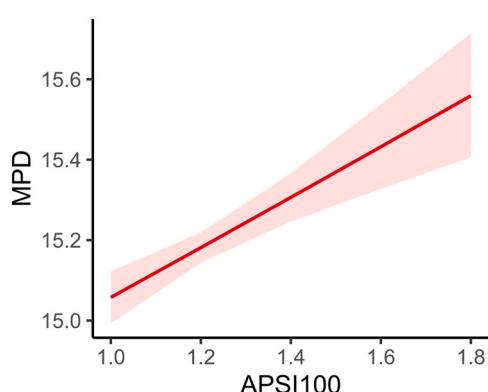
We found that landscape structure is the main driver of soil microbiome alpha and beta diversity in intensive agricultural landscapes in Cerrado. Although landscape structure affected soil chemical properties, soil physico-chemical properties could not explain variation in taxonomic, phylogenetic, and functional alpha diversity, contrary to our expectations. However, variation in soil chromium concentration among landscapes explained beta diversity.

### 4.1. Landscape structure was the main drive of microbiota taxonomic, phylogenetic and functional alpha diversity

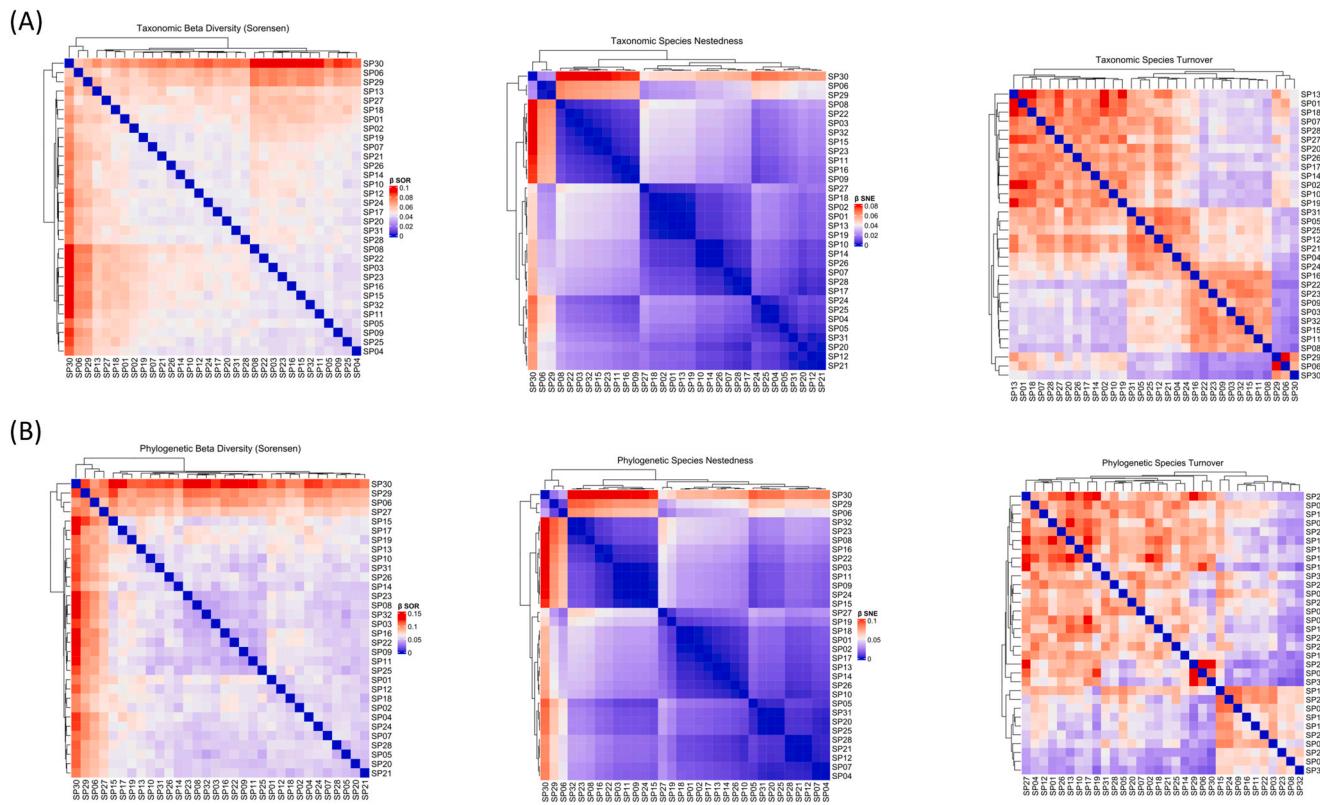
Species richness and phylogenetic diversity were mainly affected by shape (APSI) and number of patches (NP) of natural vegetation at fine spatial scales (100–200 m). Contrary to our predictions (vii), our findings showed that Archaea and Bacteria richness, and MPD (mean phylogenetic distance) increased in landscapes dominated by natural vegetation areas of irregular shape. This result agrees with a study performed in the same landscape with plant communities, where remaining patches of irregular shape had a positive effect on plant functional richness (Martello et al., 2023).

Further, landscapes dominated by natural vegetation areas of irregular shape also had a higher plant phylogenetic diversity in Cerrado (Coelho et al., 2020). Natural vegetation patches with irregular shape and angular edges (higher complexity) have smaller core areas (Laurance and Yensen, 1991), which can reduce plant functional variability (Arellano-Rivas et al., 2018). Because plant community composition affects diversity of soil microorganisms (Chu et al., 2011), the higher functional traits variation in small and irregular patches of natural vegetation may promote increased microbiota richness and phylogenetic diversity (Shigyo et al., 2019).

In addition, we found that the %pasture at 100 m and patch shape at 1000 m spatial scale positively influenced P content in soils (Fig. 4, Table S12). Although phosphorus concentration had no direct effect on species richness and phylogenetic diversity, patch shape directly influenced P concentration. This result contrasts other studies that have reported a greater association between microbiota and soil properties (Selari et al., 2021; Zhang et al., 2023; Raimbault et al., 2024; Siewe et al., 2024). However, the increase in species richness and phylogenetic diversity may be an indirect effect of the increase in phosphorus concentration. Increase in phosphorus concentration increases Bacteria and Archaea richness, especially in ecosystems with limited amount of P (Wu et al., 2022). In Brazil, P is highly used in intensive agriculture due to the small soil natural concentrations (Roy et al., 2016), particularly in intensive pasture systems (Damian et al., 2020), and in areas previously occupied by savannas (Mendes et al., 2012; Hunke et al., 2015; Withers et al., 2018). The LAND landscapes are dominated by intensive agriculture with high inputs of fertilizers that may run off to natural vegetation remnants, which are predominantly irregular and small (Santos et al., 2022), increasing P concentration and leading to higher microbial species richness and phylogenetic diversity.



**Fig. 4.** Relationship between phylogenetic diversity measured by mean phylogenetic distance (MPD) and average patch shape index (APSI) at 100 m spatial scale. Shaded area represents 95 % confidence intervals.



**Fig. 5.** Beta diversity between pairs of sampling site in LAND landscapes. (A) Taxonomic beta diversity ( $B_{\text{Sor}}$ ) and its components of nestedness ( $B_{\text{Sne}}$ ) and turnover ( $B_{\text{Sim}}$ ). (B) phylogenetic beta diversity and its components of nestedness and turnover. Colors represent different values of beta diversity, according to the figure legends.

Landscapes with a higher number of natural vegetation patches at the fine spatial scales (100–200 m) had higher microbiome species richness and functional diversity following our expectation (ii). Natural vegetation soils harbor higher richness and different species composition compared to intensive agriculture soils (e.g., Catão et al., 2014; Kumar et al., 2022; Hartmann and Six, 2023). In addition, our results pointed to an indirect effect of organic matter content on functional diversity. Organic matter content is a dominant factor to determine bacteria diversity in soils at local scale (Tian et al., 2018; Zhang et al., 2023), since these microorganisms depend on organic matter decomposition to survive (Tian et al., 2018), which explains our result. Thus, the increase in functional diversity may also be due to the higher amounts of organic matter in natural vegetation patches (Mushinski et al., 2019; Hartmann and Six, 2023). Deforestation reduces the amount of soil organic matter changing microbiome species composition. For instance, in Amazon, deforestation led to a decrease in soil organic matter and an increase in Bacteria alpha diversity, but with an increase in the relative abundance of copiotroph bacteria such as Actinomycetales, and a decrease in Chlamydiae, Planctomycetes and Verrucomicrobia (Navarrete et al., 2015). We also found high abundance of Actinobacteria in LAND agriculture landscapes, which agrees with other findings in agriculture and pasture areas in Cerrado (Quirino et al., 2009; Souza et al., 2016), also associated to degraded environments (Selari et al., 2021). Proteobacteria was the most diverse phylum in our samples. Characterized by gram-negative bacteria, Proteobacteria has high morphological diversity, and is an indicator of soil modifications due to changes in land cover (Kim et al., 2021).

In addition, we also found a negative influence of %savanna at 100 m and landscape compositional heterogeneity (SHDI) at 700 m spatial scale on soil organic matter content (Fig. 5). The different types of Cerrado vegetation harbor a gradient of vegetation structure and soil physico-chemical characteristics (Ruggiero et al., 2002). Likewise, soils

in savannas tend to have fewer nutrients than soils in seasonal forests, including organic matter content (Mendes et al., 2012). This pattern may explain the negative and strong relationship between organic matter content and %savanna found in this study. Furthermore, the savanna remnants are the smallest and most degraded natural vegetation areas in the studied landscapes.

Higher values of landscape compositional heterogeneity (SHDI) indicate greater diversity of land cover types within a landscape. A higher diversity of land covers may benefit biodiversity due to the diversity of resources delivered (Santos et al., 2021). However, in our study area, the increase in SHDI is due to the intensive production systems and human-made structures (Fig. 1B). Therefore, a higher compositional heterogeneity can negatively influence soil properties in natural vegetation areas (Gámez-Virués et al., 2015), mainly those in edge with crops, explaining the decrease in soil organic matter content.

The effects of habitat fragmentation on biodiversity are still in debate (Su et al., 2022; Galán-Acedo et al., 2024; Batsch et al., 2024). Some studies report negative effects while others report positive effects of habitat fragmentation on different taxa (De Camargo et al., 2018), mainly to non-habitat dependent species (Chetcuti et al., 2020). Our findings corroborate another study that reported a positive effect of landscape structure changes on the soil microbiota diversity (Su et al., 2022). Fragmentation increases edge effects, altering the structure, composition, moisture, and microclimate of natural vegetation (Santana et al., 2021; Yang et al., 2022; Aguiar et al., 2023), as well as, affecting soil physico-chemical and structural properties (Cardelús et al., 2020; Schedlbauer and Miller, 2022; Aguiar et al., 2023). Overall, changes in soils of natural vegetation areas influenced by alterations in landscape composition and configuration tend to benefit some soil microorganism communities, mainly those that are tolerant and tend to survive in altered environments (Su et al., 2022), explaining our results.

#### 4.2. Low beta diversity implies in a homogenization of the microbiota in the landscapes

We found low taxonomic and phylogenetic beta diversity between landscapes, and similar values of turnover and nestedness, meaning that, despite the increase in species richness and phylogenetic diversity with changes in landscape structure, species composition is very similar between landscapes. The contrast between alpha and beta diversity might be an early sign of homogenization by altering the relationship among species richness and its heterogeneity among sites (Hewitt et al., 2010). This result suggests that the LAND soil microbiota community is dominated by a species group more adapted to intensive soil management, and high inputs of nutrients and pesticides (Guan et al., 2017; Peng et al., 2024). In the Amazon Forest, for instance, soil bacterial species composition also changed following deforestation due to modifications in soil fertility (Navarrete et al., 2015). Also, in the Amazon Forest, taxonomic and phylogenetic diversity increased with the conversion of forest to pasture, but communities were more similar, due to a decrease in Acidobacteria, Nitrospirae and Gemmatimonadetes and increase in Firmicutes, Actinobacteria and Chloroflexi (Rodrigues et al., 2013).

In Sumatra, the substitution of lowland rainforest to rubber or palm oil plantations changed Bacteria species composition, shifting from the dominance of Proteobacteria groups in rainforest soils to Acidobacteria in managed soils (Schneider et al., 2015). In fact, we found a prevalence of *Bradyrhizobium* genus in our samples. Although *Bradyrhizobium* is found in both native and disturbed environments, the genus seems to be better adapted to agriculture (Procópio and Barreto, 2021). *Bradyrhizobium diazoefficiens* the most common species in our samples is a soybean N2-fixing endosymbiont (Itakura et al., 2009). The predominance of this species was expected because our sampling sites are surrounded by soybean plantations, and *B. diazoefficiens* is the most abundant rhizobial species in the soybean rhizosphere (Liu et al., 2018). *Bradyrhizobium erythrophlei*, the second most abundant species, is also highly present in soil samples associated with plant roots (Yao et al., 2015; Michel et al., 2017), and together with *B. diazoefficiens* can be found in association with soybean rhizosphere (Liu et al., 2021).

We found that taxonomic and phylogenetic beta diversity increased with the difference in %natural vegetation and chromium concentration between landscapes, following our expectations (i and v), meaning that the higher the difference in the amount of natural vegetation and chromium concentration the greater the difference in species composition. The high concentration of Cr in the LAND landscapes is most likely due to the input of phosphorus fertilizer. High levels of Cr can affect nitrification, aerobic nitrite oxidation, and microbiota metabolic function, and thus modifying species composition leading to the high abundance of heavy metal tolerant species (He et al., 2016; Li et al., 2020). For instance, *Cupriavidus metallidurans* the most abundant species in sites 15 and 23, is a heavy metal resistance species with several genetic adaptations, and a complex transcriptional network related to heavy metal metabolism (Janssen et al., 2010; Monsieurs et al., 2011). Furthermore, we found *Anaeromyxobacter* in all soil sampling sites, and it was the most abundant genus in site 32, which is predominantly occupied by an intensive system of soybean and maize plantations. *Anaeromyxobacter dehalogenans* has prominent role in reducing metals such as uranium and iron in contaminated soils (Sanford et al., 2002; Wu et al., 2006b). In addition, soil characteristics affect plant community diversity and composition, which in turn affect the soil microbiota (Dinakaran et al., 2019), which may explain the influence of %natural vegetation on beta diversity. For instance, motility and chemotaxis categories were highly abundant in sites 31 and 32, which were close to streams in a riparian forest. Motility and chemotaxis genes tend to be more abundant at less structured soils close to water courses, and with high organic matter content (Dini-Andreote et al., 2018), and play a role in multiple functions in microbes physiology, such as nutrient acquisition, expansion of population range and biofilm formation (Colin et al., 2021). Most genes in chemotaxis and motility categories decrease in

abundance with the land use conversion (Berkelmann et al., 2018, 2020).

We also found a high percentage of novelty in taxonomical and functional classification, with c. 70 % of unknown species and 50 % of unknown protein or ribosomal RNA. The percentage of taxa classification in metagenomics studies is determined mainly by database composition (Nasko et al., 2018; Ye et al., 2019). Although RefSeq database is in constant growth, with over 1000 Archaea species and almost 100,000, still these numbers are far below the number of Bacteria and Archea species yet to be discovered (Lombard et al., 2011; Garg et al., 2024).

#### 5. Conclusion

We found that intensive agriculture increases the soil microbiota taxonomic, functional, and phylogenetic alpha diversity. In contrast, it tends to decrease the soil microbiota beta diversity. Taking together, our results show that rather than decreasing Archaea and Bacteria species richness, the intensive agriculture is modifying the community's structure homogenizing species composition between landscapes, leading to a dominance of groups more adapted to agriculture soils condition.

Thus, our results highlight the urgent need of increasing habitat amount in these landscapes to increase beta diversity, minimizing community homogenization. We found few and non-significant direct effects of soil properties on the soil microbiota. Thus, our findings emphasized the stronger effect of landscape structure on the soil microbiota alpha and beta diversities. However, it is important to emphasize that we collected soil data in an unique period, which could explain this absence of significant effects of soil properties on microbiota. Furthermore, differences in chromium concentration affected both taxonomic and phylogenetic beta diversity.

All these changes will compromise the soil microbiota ecosystem functions such as nutrient cycling, thus affecting plant establishment and dispersal, as well as, crop yield. We suggest that future studies also investigate the relationship between plant functional traits, vegetation types, and soil microbiota diversity, to help define patterns of relationship between these microorganism and land cover changes in Cerrado.

#### Author contributions

RGC conceived and funded the work; LDV, DMS and MBS collected the samples and extracted DNA; LDV performed bioinformatics analysis and community analysis; LDV and RGC curated the data; LDV, JSS and EH performed statistical analyses. RGC wrote the manuscript with inputs from JSS. All authors contributed critically to the drafts and gave final approval for publication.

#### CRediT authorship contribution statement

**Rosane Garcia Collevatti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Silva Daniela M:** Investigation. **Vieira Lucas:** Investigation, Formal analysis, Data curation. **Benvindo Marcelino:** Investigation. **Erica Hasui:** Formal analysis. **Santos Juliana S:** Writing – review & editing, Formal analysis.

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## Declaration of Competing Interest

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

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2025.109807](https://doi.org/10.1016/j.agee.2025.109807).

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