

## Soil management shapes bacterial and archaeal communities in soybean rhizosphere: Comparison of no-tillage and integrated crop-livestock systems

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### ABSTRACT

Sustainable agricultural systems play a crucial role in improving soil properties and enhancing crop yields. Particularly for soybean, a vital agricultural commodity, no-tillage (NT) and integrated crop-livestock (ICL) systems have been employed in tropical regions. Despite the recognized benefits of using NT and ICL, there is a significant knowledge gap regarding their impact on the rhizosphere microbiome of soybean. Therefore, this field study aimed to explore and compare the responses of the bacterial and archaeal communities within the soybean rhizosphere in both NT and ICL systems. To address this objective, in addition to sampling the soybean rhizosphere, we collected samples from the bulk soil in the NT area and the rhizospheres of grass (*Urochloa brizantha*) and corn (*Zea mays* L.) in the ICL system, covering the typical land use in this region. The results revealed distinct bacterial and archaeal communities in the soybean rhizosphere under NT and ICL. Specifically, the ICL system enriched the soybean rhizosphere with KD4\_96 (score 3), Vicinamibacteraceae (score 3), *Candidatus Nitrocosmicus* (score 2.5), and *Methylobacterium* (score 2.5). In contrast, NT led to an enrichment of Solirubrobacter (score 3), Amycolatopsis (score 2.8), Sphingomonas (score 2.8), and Nitrososphaeraceae (score 2.5). Microbial community interactions exhibited greater complexity in the soybean rhizosphere under NT (676 nodes and 7095 edges). Notably, both bacterial and archaeal communities in the soybean rhizosphere under NT and ICL demonstrated potential functionality in nitrogen fixation. Thus, this study showed that NT and ICL promoted different responses of bacterial and archaeal communities within the soybean rhizosphere which, can influence the plant's performance.

### 1. Introduction

During the last decade, agricultural systems have placed a strong emphasis on sustainability, particularly from an environmental perspective. This emphasis includes integrated agricultural systems that introduce different production methods within the same area and

employ distinct strategies (Bieluczyk et al., 2020), such as no-tillage (NT) and intercropping systems. In Brazil, the total area under both NT and intercropping agricultural systems encompasses approximately 50 million hectares (Embrapa, 2023). These agricultural systems yield various benefits for both agriculture and the environment, such as increased productivity (Chai et al., 2021) and reduced greenhouse gas

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emissions (Hai et al., 2020). Concerning the positive impact on the soil environment, these systems contribute to an increase in soil organic matter and nutrients (Bieluczyk et al., 2020), preserve soil moisture (Glatzle et al., 2021), and create favorable conditions for soil microbial communities (Bertola et al., 2021).

The soil microbial communities play a crucial role in both agricultural production and environmental sustainability. Notably, bacterial and archaeal communities are among the most abundant and diverse microbial communities found in soils (Singh et al., 2009), playing essential roles in soil and plant productivity, particularly within integrated agricultural systems (Pratibha et al., 2023). Indeed, both bacterial and archaeal communities contribute to nutrient cycling through the decomposition of organic residues (Raza et al., 2023), being involved in various steps of nitrogen cycling (Gubry-Rangin et al., 2010), such as ammonification and nitrification (Diao et al., 2023). In addition, these communities facilitate processes such as nitrogen fixation promotion (Sepp et al., 2023) and phosphate solubilization (Tian et al., 2021). These processes, orchestrated by both bacterial and archaeal communities, make nutrients more accessible to plants, thereby promoting higher productivity (Kebede, 2021). Particularly, both bacterial and archaeal communities can help reduce gas emissions into the atmosphere, thereby mitigating the greenhouse effect (Bardgett et al., 2008; Liu et al., 2022).

Therefore, the knowledge of structure, diversity, and composition of both bacterial and archaeal communities in agricultural systems is important to increase their sustainability. NT is a well-known agricultural system that requires the maintenance of plant coverage on soil and has been recognized for its ability to enhance soil properties and increase crop yields (Busari et al., 2015; Calonego et al., 2017; Galdos et al., 2019). Over the last decade, integrated crop-livestock (ICL) has emerged as a noteworthy system that promotes plant productivity through the enhancement of nutrient cycling (Farias et al., 2020). Several studies have shown the positive effect of ICL on soil properties, increasing the soil organic matter content (Bieluczyk et al., 2020) and improving the biological properties (Sekaran et al., 2021). Regarding the effect on soil bacterial community, ICL has positively contributed to changing the community composition and diversity (Chen et al., 2018; Sarto et al., 2020; Bansal et al., 2022). For instance, Bansal et al. (2022) observed enhanced phospholipid fatty acid (PLFA), total bacterial biomass, gram (+), gram (-) bacteria in ICL as compared to conventional agriculture.

Despite its beneficial effects on agriculture, there are few studies specifically comparing NT and ICL systems regarding their impact on soil properties and crop yield (Assmann et al., 2014; Souza et al., 2016). Concerning soybean, the primary crop cultivated under both NT and ICL, it is not known how each system affects its rhizosphere and the associated microbial communities. This is a critical question as the rhizosphere of plants is influenced by soil management, and the rhizospheric microbial communities are in turn affected by the rhizosphere (Leite et al., 2024). For instance, a recent study by Pires et al. (2021) observed higher mycorrhiza diversity and richness in the rhizosphere of soybean intercropped with *Urochloa* under ICL.

Therefore, we hypothesized that the rhizosphere of soybean under the ICL system could show distinct diversity and composition of bacterial and archaeal communities, differing from those observed in soybean under NT. Thus, this study is designed to evaluate the bacterial and archaeal communities in the rhizosphere of soybean under the ICL system, particularly when intercropped with grasses and compare them with the rhizosphere of soybean under the NT system. For this, we assessed the microbial communities through the sequencing of the 16S rRNA gene and correlated them with chemical and biological soil parameters. With this, we aimed to gain insights into how more sustainable agricultural practices benefit the recruitment of beneficial microbes in the rhizosphere of soybean, potentially influencing plant growth and health.

## 2. Material and methods

### 2.1. Field location

The study was carried out from March 2022 to April 2023 at Fazenda Barbosa, Brejo, MA, Brazil (3°42'1.66"S; 42°56'25.08"W; 102 m). The soil is classified as Yellow Argisol Typical Dystrocohesive (PAdx), with a predominance of kaolinite (Dantas et al., 2014), and presents 76% sand, 8% silt, and 16% clay. The climate is tropical (Aw) according to the Köppen-Gerger (Brito et al., 2023). The average annual precipitation and temperature are 1,613 mm and 27 °C, respectively (Maranhão, 2022).

### 2.2. Agricultural systems

We selected two main agricultural crop systems: no-tillage (NT) and integrated crop-livestock (ICL) (Fig. 1). The NT area was deforested in 2003, with the application of 2 tons ha<sup>-1</sup> of calcitic limestone, and it has been annually cultivated with soybean and millet under crop succession. Each year, soil fertilization consists of 100 kg ha<sup>-1</sup> of ammonium sulfate, 150 kg ha<sup>-1</sup> of monoammonium phosphate, and 170 kg ha<sup>-1</sup> of potassium chloride. The ICL area was deforested in 2009, with the application of 1 ton ha<sup>-1</sup> of dolomitic limestone +0.5 ton ha<sup>-1</sup> of gypsum. The no-tillage system was implemented with soybean and millet under crop succession until 2020. Starting from 2021, soybean has been cultivated followed by pasture (*Urochloa brizantha* cv. Marandu) and intercropped with corn. In both systems, soybean is sown at a population of 300,000

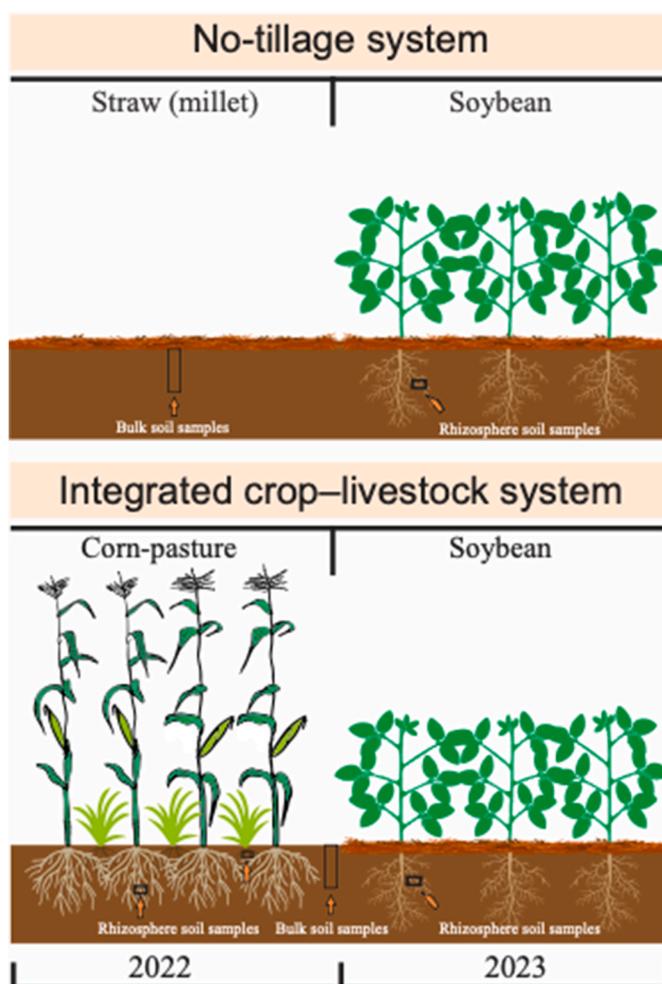


Fig. 1. Illustration depicting the variances in agricultural management systems.

plants per hectare. Annually, soil fertilization consists of 340 kg ha<sup>-1</sup> (formulation NPK 12-30-00) plus 200 kg ha<sup>-1</sup> (formulation NPK 10-00-30) after 30 days. After the crop harvest (soybean and corn), the pasture is grazed by cattle (three units of animals per hectare).

### 2.3. Rhizosphere sampling

In both NT and ICL systems, the sampling of soybean rhizosphere was conducted in March 2023. However, to evaluate the effect of both NT and ICL systems on bacterial communities in the rhizosphere of soybean, we collected samples from bulk soil, and the rhizosphere of grass (*Uruchloa brizantha*) and corn (*Zea mays* L.) in ICL. To collect the rhizosphere samples, the soil adhered to roots was carefully collected and put into sterile Eppendorf tubes, which were immediately frozen at a temperature of -80 °C. The bulk soil was collected at 0–10 cm depth and stored in sterile bags at -80 °C. Both bulk soil samples from NT and ICL were chemically and biologically analyzed according to Teixeira et al. (2017) and the results are shown in Table S1. Briefly, soil pH was determined in a 1:2.5 soil/water extract. Available phosphorus (P) and potassium (K) were estimated by photometry. Total organic carbon (TOC) was estimated according to Yeomans and Bremner (1988). Microbial biomass C (MBC) was assessed according to Vance et al. (1987). The activities of fluorescein diacetate hydrolysis (FDA), urease, dehydrogenase, and acid phosphatase were assessed according to Tabatabai (1994).

### 2.4. DNA extraction and sequencing

To extract DNA, 0.5 g of soil samples were used with a DNA Isolation Kit (DNeasy PowerSoil Pro Kit; Qiagen, CA, USA). The quality and concentration of DNA were measured using a spectrophotometer. The primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVHHHTWTCTAAT-3'), were used to amplify the V4 region of the 16S rRNA by polymerase chain reaction (PCR) (Caporaso et al., 2011). PRC program was: 95 °C for 3 min, followed by 35 cycles at 98 °C for 20 s, 55 °C for 30 s, 72 °C for 30 s, and 72 °C for 5 min. This procedure includes sequencing adapters and dual-index barcodes to differentiate each sample. PCR products were purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) and quantified by Qubit fluorometer with dsDNA BR Assay kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The libraries were paired-end sequenced using the Illumina MiSeq Reagent Kit v2 (300-cycles, 2 × 150 bp) (Illumina) at the Genomic and Bioinformatic Facility Centre (CeGenBio) of the Federal University of Ceará, Brazil.

The 16S rRNA sequences were preprocessed using the QIIME 2 v2021.11 software. Demultiplexing and quality control were conducted using DADA2 (Callahan et al., 2017), employing the consensus method to eliminate any remaining chimeric and low-quality sequences (<q20). The 16S rRNA sequencing approach yielded approximately 11,409,000 reads, averaging 475,370 sequences per sample. These sequences were then rarefied to 252,750 sequences, based on the lowest sample count, and singleton and doubleton sequences were removed. Taxonomic classification for the 16S rRNA region was done using the SILVA database version 138 at 97% similarity (Quast et al., 2013). The 16S rRNA data are available at NCBI SRA under the identification PRJNA1069081.

### 2.5. Data analysis

To compare the structure of bacterial and archaeal communities and its correlation with environmental parameters, Principal Component Analysis (PCA) and Redundancy Analysis were employed using Canoco 5 (Biometrics, Wageningen, The Netherlands). Furthermore, a permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was performed to identify significant differences in bacterial community structures among the samples. Subsequently, microbial composition was compared using the LDA Effect Size (LEfSe) analysis, as

outlined by Segata et al. (2011). For this analysis, the OTU table at the phylum, genus, and OTU levels was used to assess community composition. A functional prediction annotation was carried out to evaluate the potential functions exhibited by both bacterial and archaeal communities using PICRUSt2 (Douglas et al., 2020), with a focus on the nitrogen cycle. Finally, we assessed the complexity of community interactions through network analysis. SparCC correlations (Friedman and Alm, 2012) were calculated, and only significant ( $p < 0.01$ ) and strong ( $>0.9$  or  $< -0.9$ ) correlations were selected. For network visualization and the calculation of topological properties, we utilized the interactive platform Gephi (Bastian et al., 2009).

## 3. Results

### 3.1. Structure of bacterial and archaeal communities

The results of PCA explained 18.38% of total variation and revealed a distinct clustering of bacterial and archaeal communities between NT and ICL (Permanova  $F = 2.809$ ,  $P = 0.0001$ ; Fig. 2A). Notably, the bacterial and archaeal communities in the rhizosphere of soybean under NT clustered with the communities found in bulk soil. In the ICL, the bacterial and archaeal communities in the soybean rhizosphere were distinctly separated from those found in rhizospheres of grass and corn, which clustered together. The RDA analysis comparing only rhizospheres and its correlation with environmental parameters explained 20.36% of the total variation and showed that bacterial and archaeal communities in soybean from both NT and ICL were clustered apart from the rhizosphere of corn and grass, as shown in the first axis of the RDA (Permanova  $F = 2.51$ ,  $P = 0.0001$ ; Fig. 2B). Also, the samples from the rhizosphere soybean were different between NT and ICL. The community of the soybean rhizosphere under no-tillage correlated with soil microbial biomass and enzymes, while the samples from grass and corn correlated with fluorescein diacetate and microbial quotient.

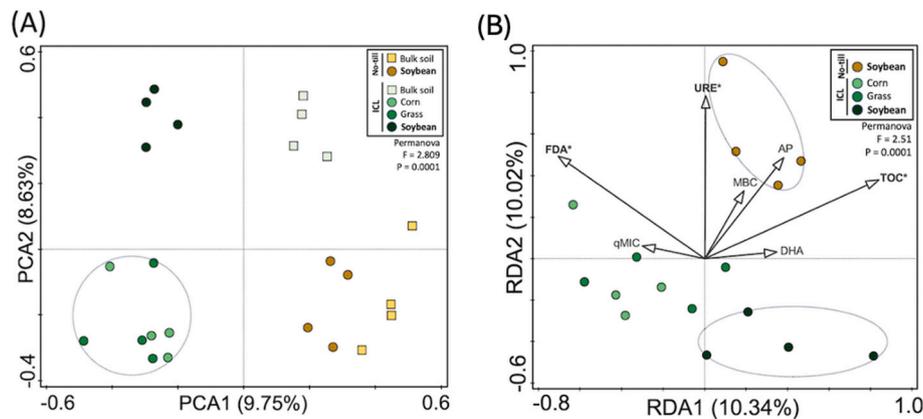
### 3.2. Composition of bacterial and archaeal communities

The top three most abundant microbial phyla identified in bulk soil and the rhizosphere in both agricultural systems were Actinobacteriota (30% of the total sequences), followed by Proteobacteria (19%), and Firmicutes (10%) (Fig. S1A). Comparing bulk soil and soybean rhizosphere, the LDA analysis showed that the soybean rhizosphere increased the abundance of Proteobacteria and Bacteroidota, while Acidobacteriota, Chloroflexi, and Crenarchaeota phyla were more abundant in the bulk soil ( $P < 0.05$ , Fig. S1B). The rhizosphere of corn and grass presented a higher abundance of Actinobacteriota and Myxococcota, respectively ( $P < 0.05$ , Fig. S1C).

We then examined the enrichment of bacterial and archaeal groups at the genus and OTU levels (Fig. 3). The heatmap illustrates the specific enrichment of OTUs for each treatment, revealing a distinct pattern between the rhizosphere of soybean under NT and ICL (Fig. 3A). This analysis unveiled that the rhizosphere of soybean under ICL exhibited an increase in 21 taxa, while the rhizosphere of soybean under NT showed an increase in 14 taxa. More specifically, a comparison of the rhizosphere community of soybean under the two distinct systems highlighted an enrichment of KD4\_96, Vicinamibacteraceae, *Candidatus Nitrocosmicus*, and *Methylobacterium* in ICL, whereas under NT, the rhizosphere of soybean demonstrated an enrichment of *Solirubrobacter*, *Amycolatopsis*, *Sphingomonas*, and *Nitrososphaeraceae* ( $P < 0.05$ , Fig. 3B).

### 3.3. Interactions between bacterial and archaeal groups

The co-occurrence network analysis showed a distinct pattern of bacterial and archaeal interactions in bulk soil and rhizosphere comparing both NT and ICL (Fig. 4; Table S2). In NT, both bulk soil and rhizosphere of soybean showed a similar number of nodes (671 and 676 nodes, respectively, in bulk soil and rhizosphere). However, the



**Fig. 2.** Structure of the bacterial and archaeal community in bulk soil and rhizosphere associated with crops in no-tillage (No-till) and integrated crop-livestock (ICL) systems based on the 16S rRNA gene. **(A)** Principal component analysis (PCA) including samples from bulk soil and rhizosphere. **(B)** Redundancy analysis (RDA) of the rhizosphere in the two evaluated systems. Arrows indicate the correlation between microbial profile, organic carbon, and soil microbial properties. The analysis of permutation (PERMANOVA) is indicated in the top right corner of the graphs. TOC: total organic carbon; MBC: microbial biomass carbon; qMIC: microbial quotient; DHA: dehydrogenase; FDA: fluorescein diacetate; URE: urease; AP: acid phosphatase.

bacterial and archaeal communities in the rhizosphere of soybean, under no-tillage, presented a higher number of edges (4442 and 7095 edges, respectively, in bulk soil and rhizosphere). In the integrated crop-livestock, bulk soil (568 nodes and 2896 edges) and the rhizosphere of corn (569 nodes and 2637 edges) showed a lower complexity compared to the rhizosphere of grass (676 nodes and 4493 edges) and soybean (628 nodes and 4028 edges). Comparing soybean under NT and ICL, we observed a higher complexity in the rhizosphere of soybean in the NT (676 nodes and 7095 edges). In addition, the bacterial and archaeal communities in the rhizosphere of soybean under NT showed higher values of average degree (Table S2).

### 3.4. Functional prediction profiling

Since both NT and ICL aim to increase and maintain soil sustainability through organic matter, we predict potential functions associated to N cycling (Fig. 5). We observed distinct responses of potential functions comparing soybean under NT and ICL. The potential functions related to organic degradation and nitrification were observed in both bulk soils. However, the bacterial and archaeal communities in the rhizosphere of soybean under NT showed potential functions related to nitrogen organic degradation. In addition, both bacterial and archaeal communities in the rhizosphere of soybean under NT and ICL showed potential functions on N fixation. Interestingly, a higher abundance of genes related to assimilatory nitrate reduction was observed in the rhizosphere as compared to bulk soils in both systems.

## 4. Discussion

### 4.1. Structure of bacterial and archaeal communities

The results of this study confirmed the hypothesis that the rhizosphere of soybean in both no-tillage and integrated crop-livestock systems exhibited distinct bacterial and archaeal communities and their interactions. This suggests that, despite being the same crop species, the rhizosphere composition is influenced by the specific agricultural system (J. Chen et al., 2022). The bacterial and archaeal communities in the rhizosphere of soybean differed between no-tillage and integrated crop-livestock systems. In the no-tillage system, the bacterial and archaeal communities in the soybean rhizosphere were closely related to those in the bulk soil, indicating an influence of millet straw added to the soil surface. This aligns with previous studies that have reported the impact of straw returning on microbial communities in bulk soil, which are then recruited by the rhizosphere (Bu et al., 2020). Additionally,

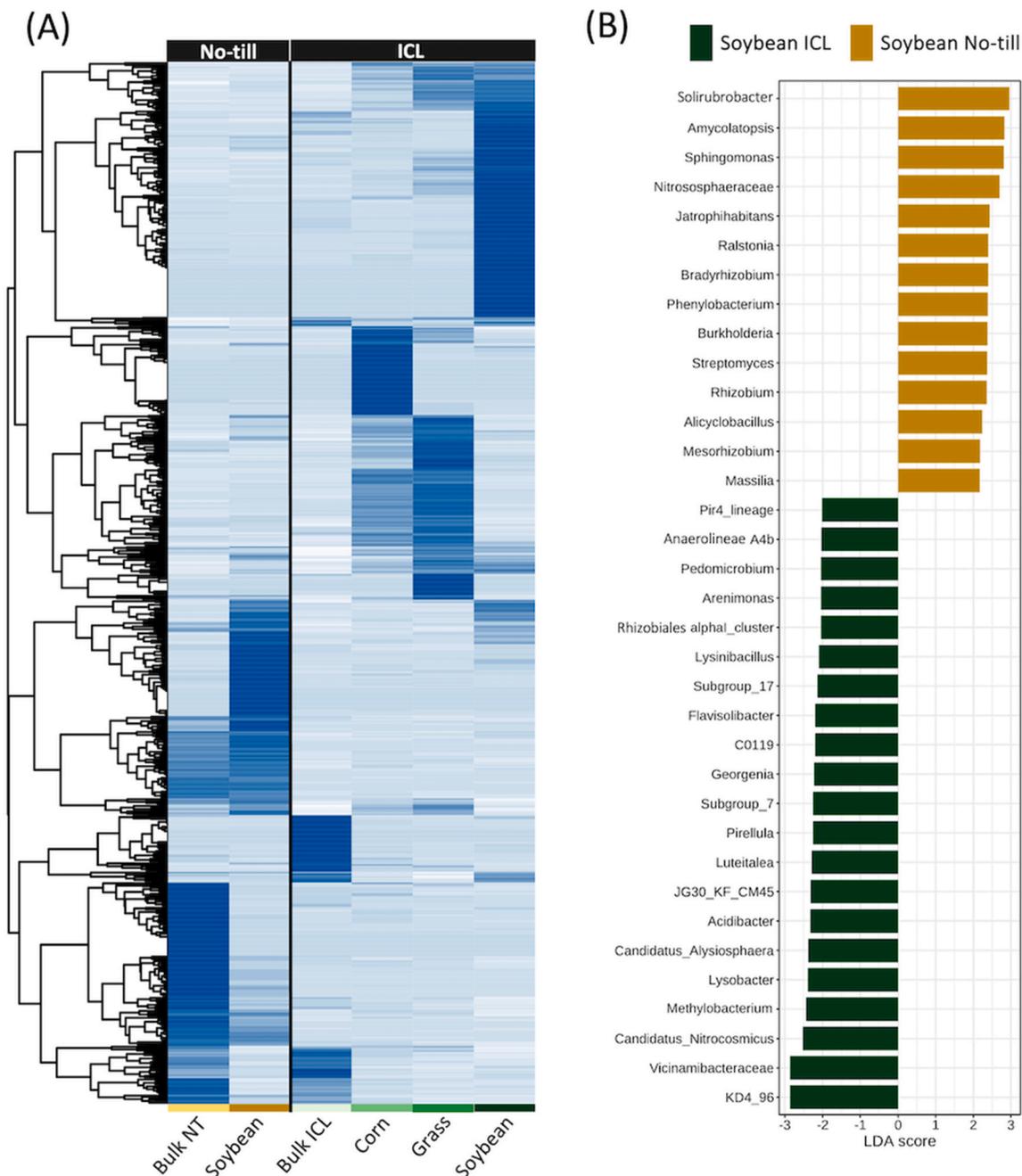
Ridder-Duine et al. (2005) reported that bacterial communities in the rhizosphere are generally more similar to those in the bulk soil than to other rhizosphere communities. This may explain why the rhizosphere of soybean under integrated crop-livestock systems was not influenced by the microbial communities associated with both grass and corn rhizospheres.

The distinct characteristics of plant species within integrated crop-livestock systems, particularly grass and corn (Poaceae family), may contribute to differences in the bacterial and archaeal communities present in the soybean rhizosphere (Leguminosae family). Regarding the effect of microbial properties, the presence of grass stimulates microbial biomass due to a higher proportion of C than N, thus increasing the microbial population (Amorin et al., 2020). In contrast, the presence of legumes promotes more C and N mineralization, thereby decreasing microbial biomass. When the bacterial community was considered, a previous study observed that the rhizosphere of corn (Poaceae) and cowpea (Leguminosae) in subsequent cultivation were distinct, suggesting an effect of plant species and demonstrating that the microbiome from distinct rhizospheres was not shared (Araujo et al., 2019). This effect is attributed to differences in root exudation between plant species, where Poaceae family plants typically release carbohydrates, while leguminous plant species release amino acids (Merbach et al., 1999; Alvey et al., 2003). In addition, a previous study has shown that legumes plant species present highest amount of rhizosphere carboxylates than grasses (Kidd et al., 2016). Interestingly, when comparing rhizospheres, the prokaryotic community in soybean under no-tillage was associated with soil organic C, microbial biomass, and enzymes, suggesting a direct effect of millet straw as the main C source for soil microorganisms (Zhang et al., 2019).

### 4.2. Composition of bacterial and archaeal communities

In general, we observed a high relative abundance of Actinobacteria, Proteobacteria, and Firmicutes, as expected in both agricultural systems. This prevalence can be attributed to their role in facilitating the maintenance of organic carbon, primarily through the input of straw and root debris, consequently fostering the proliferation of these microbial groups (S. Chen et al., 2022). The high abundance of these three phyla in both agricultural systems is interesting, as members of these microbial groups play essential roles in the soil-plant continuum. They act as decomposers of organic residues, releasing nutrients, and function as plant-growth promoters, thereby enhancing plant performance (Spain et al., 2009; Bao et al., 2021).

Although Actinobacteria, Proteobacteria, and Firmicutes have

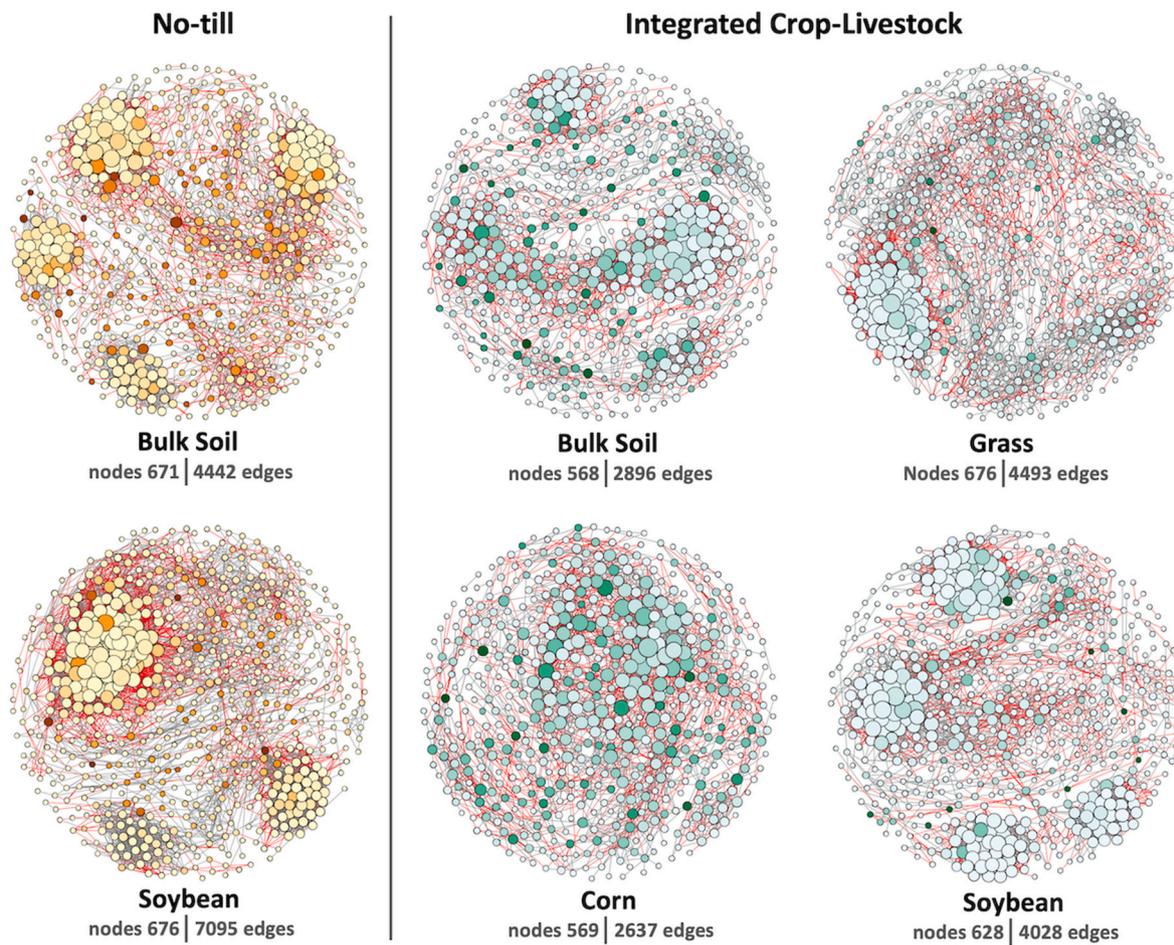


**Fig. 3.** Microbial community composition in bulk soil and the rhizosphere of in bulk soil and rhizosphere of crops in no-tillage (No-till) and integrated crop-livestock (ICL) systems. **(A)** Heatmap showing the differential abundance of OTUs among all treatments. **(B)** Pairwise comparison using Linear discriminant analysis (LEfSe) of the main microbial genera enriched in the soybean rhizosphere contrasting the evaluated systems. In all graphs, only significant different OTUs are shown ( $P < 0.05$ ).

dominated the microbial community, specific bacterial and archaeal groups were enriched in the rhizospheres of plants, particularly in soybean under no-tillage and integrated crop-livestock systems, confirming the observed pattern in the structure of bacterial and archaeal communities. Specifically, in the rhizosphere of soybean under integrated crop-livestock, there was an enrichment of KD4\_96, Vicinamibacteraceae, *Candidatus Nitrocosmicus*, and *Methylobacterium*. Notably, the enrichment of *Methylobacterium* in the soybean rhizosphere is significant, given its recognized role as an important nitrogen fixer and hormone producer (Grossi et al., 2020). Previous studies have observed *Methylobacterium* associated with the rhizosphere of soybean under sustainable farming systems (Bender et al., 2022; Agyekum et al., 2023). Additionally, we can highlight the enrichment of *Candidatus Nitrocosmicus*, a recognized aerobic ammonia oxidizer (Alves et al., 2019),

which can contribute to nitrogen cycling in integrated crop-livestock.

The rhizosphere of soybean under no-tillage enriched bacterial groups involved in C and N cycling, such as *Sphingomonas* and members of the family Nitrososphaeraceae (Dong et al., 2017; Behnke et al., 2021). The presence of straw in the no-tillage system could explain the enrichment of these specific groups, such as *Sphingomonas*, known as plant growth promoters through nitrogen fixation, contributing to soybean growth (Luo et al., 2020; Alipour Kafi et al., 2021). Regarding Nitrososphaeraceae, previous studies have reported this nitrifying microorganism impacting nitrogen cycling (Tourna et al., 2011), especially during soybean growth (He et al., 2014), contributing to an increase in nitrogen availability to plants.



**Fig. 4.** Network co-occurrence analysis of the bacterial communities in soils under a no-tillage (No-till) and integrated crop-livestock system. A connection stands for SparCC correlation with magnitude  $>0.7$  (positive correlation–gray edges) or  $<-0.7$  (negative correlation–red edges) and statistically significant ( $P < 0.05$ ). Each node represents taxa at OTU level and the size of node is proportional to the number of connections (that is, degree). The color of the nodes is based on the betweenness centrality, where darker colors indicated higher values.

#### 4.3. Bacterial and archaeal community dynamics

In general, we observed a more complex network of interactions among bacterial and archaeal communities in both bulk soil and the rhizosphere of soybean in the no-tillage system. This high number of microbial interactions in the bulk soil suggests a higher soil microbial stability in no-tillage systems, which may contribute to the assembly of the soybean rhizosphere microbiome (Gu et al., 2022). In addition, the higher average degree in the co-occurrence network observed in the rhizosphere of soybean under no-tillage suggests higher inter-dependence among bacteria and archaea and less divergent functional groups (Guo et al., 2022). Indeed, the rhizosphere of soybean under no-tillage showed higher bacteria and archaea interactions, which contributes to improving soybean growth through biological control of pathogens, production of plant regulators, and biological N fixation (Volpiano et al., 2022). The heightened complexity of the microbial community may mitigate pathogen invasion by enhancing competition for resources and niche occupancy, thereby promoting greater efficiency (Mendes et al., 2023).

In contrast, the integrated crop-livestock exhibited variations in microbial interactions according to distinct plant rhizospheres. This variability suggests plant-specific microbial dynamics within integrated crop-livestock systems, potentially affecting the network of interactions among bacterial and archaeal communities. For instance, Schmidt et al. (2019) assessed microbial communities in various plant species and agricultural management practices and observed that plant selection

drives rhizosphere microbial communities more significantly than agricultural management practices. This means that, despite integrated crop-livestock being a sustainable system, distinct rhizosphere microbial communities from different plant species, such as grass and corn, did not necessarily contribute to an increase in microbial interactions within the soybean rhizosphere.

#### 4.4. Functional prediction profiling

It is known that both no-tillage and integrated crop-livestock systems aim to increase and maintain the soil organic matter (Bieluczyk et al., 2020). Since organic matter is a primary source of nitrogen (N), we predicted potential functions related to N cycling to compare no-tillage and integrated crop-livestock systems. The results revealed contrasting responses of predicted functions related to N in the rhizosphere of soybean under no-tillage and integrated crop-livestock, indicating that distinct agricultural management practices promote potentially different effects on soil and rhizosphere functionality. In bulk soil, the potential functions related to organic degradation and nitrification indicate that the presence of organic matter in the soil stimulates biological processes related to N mineralization, thereby increasing N availability to plants (Hoffland et al., 2020).

Comparing samples from the rhizosphere across different systems, soybean under no-tillage exhibited potential functions related to organic degradation, suggesting that microbial communities play a role in organic matter decomposition (Khan et al., 2023). This could result from

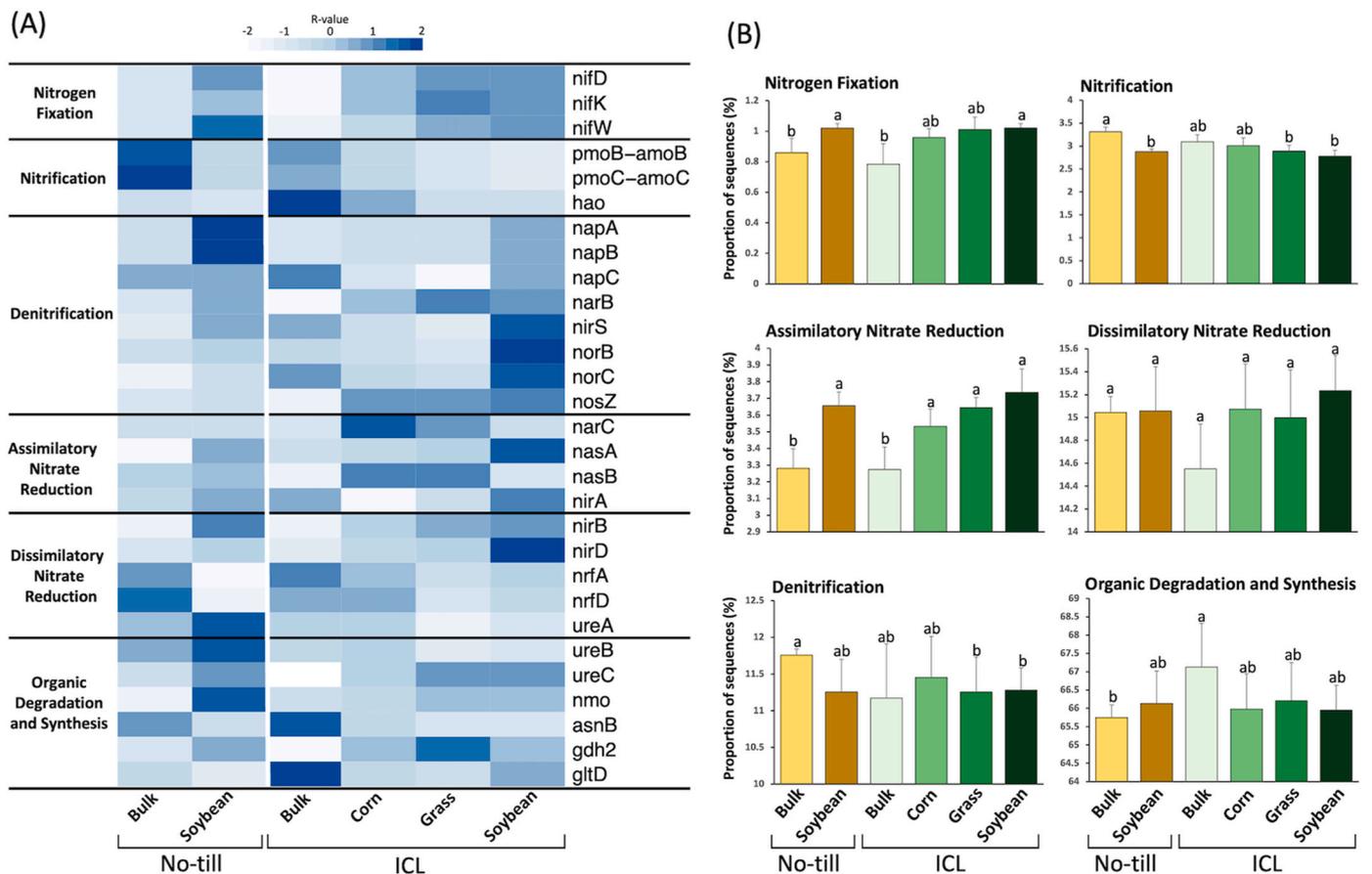


Fig. 5. (A) Heatmap and (B) bargraph showing the differential abundance of predicted functions related to nitrogen cycle in no-tillage (No-till) and integrated crop-livestock (ICL). The classification is based on the functional prediction on the FAPROTAX database. Different letters refer to significant differences ( $P < 0.05$ ) based on post-hoc Tukey-Kramer test followed by Benjamini-Hochberg FDA correction.

a similar composition of microbial communities and their interactions observed in the bulk soil and rhizosphere of soybean under no-tillage. On the other hand, both bacterial and archaeal communities in the rhizosphere of soybean under no-tillage and integrated crop-livestock systems showed potential functions related to nitrogen (N) fixation, which is important as this process makes N available from the atmosphere to plants, particularly soybean (Ciampitti et al., 2021). Interestingly, a higher potential for nitrate reduction was observed in the rhizosphere compared to bulk soils in both systems, suggesting that plant-microbe interactions in the rhizosphere can contribute to increasing the assimilation of nitrate, potentially influencing N availability for the plants (Moreau et al., 2019).

## 5. Conclusions

This study presents novel findings regarding the rhizosphere microbiome in sustainable agricultural management systems, demonstrating that no-tillage (NT) and integrated crop-livestock (ICL) practices elicit distinct responses from bacterial and archaeal communities in the soybean rhizosphere. These results underscore the significant influence of the microbiome present in the soil, such as bulk soil in NT and the rhizospheres of pasture and corn in ICL, on the rhizosphere of soybean. Consequently, the study reveals that the assembly of the rhizosphere microbiome is strongly associated with the soil management system, regardless of whether the main crop belongs to the same plant species.

In addition to highlighting the intricate interplay between soil management practices and the rhizosphere microbiome composition, our study underscores the importance of considering the broader

ecosystem dynamics in sustainable agriculture. Understanding how different agricultural practices shape microbial communities can lead to more effective management strategies that optimize crop productivity while promoting environmental sustainability.

Further studies could elucidate the specific mechanisms by which different soil management practices influence the rhizosphere microbiome and how these microbial communities, in turn, impact plant health and productivity. By gaining a more comprehensive understanding of these dynamics, we can continue to refine agricultural practices to ensure long-term sustainability and resilience in food production systems.

## CRedit authorship contribution statement

**Romario Martins Costa:** Writing – review & editing, Writing – original draft, Investigation. **Mayanna Karlla Lima Costa:** Writing – review & editing, Writing – original draft, Investigation. **Sandra Mara Barbosa Rocha:** Writing – review & editing, Writing – original draft, Investigation. **Marcos Renan Lima Leite:** Writing – original draft, Methodology. **Francisco de Alcantara Neto:** Writing – review & editing, Writing – original draft, Visualization. **Henrique Antunes de Souza:** Writing – review & editing, Writing – original draft, Visualization. **Arthur Prudencio de Araujo Pereira:** Writing – review & editing, Writing – original draft, Formal analysis. **Vania Maria Maciel Melo:** Writing – review & editing, Writing – original draft, Formal analysis. **Erika Valente de Medeiros:** Writing – review & editing, Writing – original draft. **Lucas William Mendes:** Writing – review & editing, Writing – original draft, Formal analysis. **Ademir Sergio Ferreira Araujo:** Writing – review & editing, Writing – original draft,

Supervision.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rhisph.2024.100886>.

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