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Soil bacterial community response to rhizoma peanut incorporation into Florida pastures

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Abstract

Incorporating legumes is one option for improving pasture fertility, sustainability, and biodiversity. Diazotrophic microorganisms, including rhizobia that form symbioses with legumes, represent a small fraction of the total soil microbial community. Yet, they can offset nitrogen (N) fertilizer inputs through their ability to convert atmospheric N₂ into plant-usable N via biological N₂ fixation (BNF). This study used amplicon sequencing of 16S rRNA genes to investigate soil bacterial community composition and diversity in grazed 'Argentine' bahiagrass (*Paspalum notatum* Flüge) pastures where N fertilizer was supplanted with legume-derived N from BNF in some treatments. Treatments consisted of bahiagrass fertilized with (a) mineral N (224 kg N ha⁻¹ yr⁻¹), (b) combination mineral N (34 kg N ha⁻¹ yr⁻¹) and legume-derived N via cool-season clover (CSC) (*Trifolium* spp.) mix, or (c) combination mineral N (34 kg N ha⁻¹ yr⁻¹) and legume-derived N via CSC mix and strips of Eco-turf rhizoma peanut (*Arachis glabrata* Benth.). *Bradyrhizobium* spp. relative abundance was 44% greater in the mixed pasture. Other bacterial genera with BNF or denitrification potentials were greater in pastures with legumes, whereas sequences assigned to genera associated with high litter turnover were greater in bahiagrass pastures receiving only mineral N. Soil bacteria alpha diversity was greater in pastures receiving 34 kg ha⁻¹ yr⁻¹ N fertilizer application and the CSC mix than in pastures with the CSC mix and rhizoma peanut strips. Our results demonstrate soil microbial community shifts that may affect soil C and N cycling in pastures common to the southeastern United States.

1 | INTRODUCTION

Soil N strongly influences soil bacterial community composition in grasslands (Fierer et al., 2012; Leff et al., 2015; Zeng et al., 2016). Additionally, plant species may selectively shape soil microbial communities through root exudates (Berg &

Smalla, 2009; Lundberg et al., 2012; Millard & Singh, 2010). Zhou et al. (2017) reported greater soil bacterial diversity and variation in bacterial community composition across legume species than across grass species, concluding that legume species were more influential upon soil bacterial communities than grass species. For example, symbiotically associated rhizobia that provide biological N₂ fixation (BNF) can supply over 20% of N used by grass species when grown with

Abbreviations: ASV, amplicon sequence variant; BNF, biological N₂ fixation; CSC, cool-season clover; SRS, scaling with ranked subsampling.

legumes and support greater herbage productivity as well as legume persistence over time (Jaramillo et al., 2018; Santos et al., 2018; van der Heijden et al., 2006, 2008).

Relatively little has been reported on grassland soil bacterial diversity and composition dynamics that include perennial legumes. In comparison, there are several reports addressing the soil microbiome of temperate, upland grasslands (e.g., McCaig et al., 1999; Millard & Singh, 2010) and even a few on subtropical, perennial grasslands, including bahiagrass (*Paspalum notatum* Flüggé) (e.g., Beule et al., 2019; Zhou et al., 2019). Bahiagrass is of particular interest because it dominates Florida pastures.

In North Florida, bahiagrass pasture management is often represented by mineral N fertilizer applications in the summer and overseeding with cool-season annual forages during the dormant winter months. However, N fertilizer losses from Florida pastures through leaching and subsurface runoff can contribute to Florida groundwater pollution (Silveira & Kohmann, 2020). Although bahiagrass tolerates low N fertilizer management (56 kg N ha⁻¹ yr⁻¹), greater herbage accumulation is attained at higher (180 kg N ha⁻¹) annual application rates (Mylavarapu et al., 2009; Silveira et al., 2015; Vendramini et al., 2013). A more sustainable balance between economic and environmental benefits might be realized in grass pastures with inclusion of a greater proportion of legume-derived N (Muir et al., 2011; Rouquette & Smith, 2010; Silveira et al., 2014).

Legumes added to bahiagrass pastures through overseeding cool-season cover crop mixes, including clover (*Trifolium* spp.), contribute N to the system and maintain pasture productivity until bahiagrass summer growth resumes (Dubeux et al., 2019). Additionally, incorporating a warm-season perennial legume, such as rhizoma peanut (*Arachis glabrata* Benth.), into bahiagrass pastures minimizes the need for mineral N applications and improves forage nutritional value (Dubeux et al., 2017; McCormick et al., 2014; Mullenix et al., 2016; Santos et al., 2018).

Soil microbial communities associated with monocultures of either rhizoma peanut or bahiagrass share some of the same bacterial genera (Beule et al., 2019; Wang et al., 2019). For example, *Bradyrhizobium* was the dominant bacterial genera in the soil of both plant species grown as monocultures (Beule et al., 2019; Wang et al., 2019). In mixed perennial ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) stands, soil bacteria community-level physiological profiles indicated a community composition shift, whereby the capacity to utilize different carbon sources was diminished relative to clover monocultures receiving no N fertilizer or grass monocultures receiving N fertilizer (van Eekeren et al., 2009). Using 16S ribosomal RNA (rRNA) sequence analysis, McCaig et al. (1999) observed no differences in total soil bacterial diversity but greater diversity within the Alphaproteobacteria class in an unfertilized peren-

Core Ideas

- Soil bacterial community composition in bahia-grass pastures was altered by N management.
- Legume incorporation promoted relative abundance of N-cycling bacteria.
- Soil bacteria alpha diversity did not coincide with vegetation diversity.

ennial pasture dominated by *Agrostis capillaris* L. and *Festuca ovina* L. compared with improved pastures consisting of an N-, P-, and K-fertilized *L. perenne* and *T. repens* mix. Many sequences within the Alphaproteobacteria class were closely related to *Bradyrhizobium* and *Rhizobium* spp. The presence of legumes in grasslands favors an increased proportion of the soil bacterial community being represented by rhizobia performing BNF, yet the effects of legume incorporation on soil bacterial community diversity are less clear. Changes within the soil bacterial community in response to N management and plant species may also have implications for litter turnover and nutrient cycling in perennial pastures.

In this study, we compared bahiagrass pastures under different N fertilization management, where annual mineral N fertilizer applications were offset with proportionately more BNF-N from legumes, in order to determine potential impacts on soil microbial community diversity and composition. An amplicon sequencing based account of bacterial community response to treatments at this site will help identify biological linkages to soil C and N cycling in warm-season, perennial pastures and contribute to the emerging understanding of interactions among plants, soil, and microbes. We hypothesized there would be greater soil bacterial diversity as well as a greater abundance of N cycling bacteria, including rhizobia, in treatments with BNF legumes. The objectives of this study were (a) to determine if changes occurred in soil bacterial diversity among treatments and (b) to identify which bacterial taxa were either enriched or depleted with changes in pasture N management.

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

The study site was located at North Florida Research and Education Center (NFREC), Mariana, FL (30°52' N, 85°11' W; 35 m asl). Soils were Orangeburg loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) (Soil Survey Staff, 2019). Long-term (1987–2017) average precipitation

treatment arrangement is shown in Figure 1, and a detailed description of experimental site, management, and treatment was provided by Jaramillo, Dubeux, Sollenberger, Vendramini, et al. (2021).

2.2 | Grazing management

Grazing management across treatment pastures was similar, where two tester Angus crossbreed steers (*Bos* spp.) grazed each pasture throughout the season. Put-and-take cattle of similar breed, weight, and age were assigned to pastures as needed based on target herbage allowances of 1 kg dry matter kg^{-1} body weight during the cool season and 1.5 kg dry matter kg^{-1} body weight during the warm season. A detailed description of grazing management in this study is provided by Jaramillo, Dubeux, Sollenberger, Vendramini, et al. (2021).

2.3 | Soil sampling, DNA extraction, and library preparation

In April 2017, five soil cores (diameter, 2.54 cm; depth, 10 cm) were collected from five locations within each replicated treatment. In pastures under the strip-planted treatment, five cores were collected from bahiagrass strips and five from rhizoma peanut strips. Independent soil cores were placed in sealable plastic bags and stored at 4 °C while in the field. Upon return to the laboratory within the same day, soil samples were immediately passed through a 2-mm sieve to remove large roots and debris and stored at -20 °C. Soil DNA extraction was performed using a commercial DNA isolation kit (PowerSoil, Mobio Laboratories Inc.) using 0.45 g soil. The V4 region of bacterial 16S rRNA genes was targeted in a three-step polymerase chain reaction using the primer pair 515F (5'-GTGC CAGC MGCC GCGG TAA-3') and 806R (5'-GACT ACHV GGGT WTCT AAT-3') (Caporaso et al., 2011) as described previously (Chen et al., 2018). Amplicons were indexed using 10-bp-long barcodes and zero to five frameshifting nucleotides to ensure nucleotide diversity during sequencing. Indexed polymerase chain reaction products were pooled equimolar and sequenced in one multiplexed Illumina run using the MiSeq Reagent Kit v2 (2 × 250 bp) (Illumina Inc.) at Duke Center for Genomic and Computational Biology.

2.4 | Bioinformatic processing of sequencing data

A total of 1,122,767 raw sequence reads were demultiplexed and imported in QIIME 2 version 2019.10 (Bolyen et al., 2019). Sequencing adapters and primers were removed

using cutadapt (Martin, 2011), and read quality was assessed using the 'q2-demux' plugin. Forward and reverse reads were truncated to 220 and 195 bp, respectively; quality-filtered; and merged. Chimera and singletons were removed using DADA2 (Callahan et al., 2016). Amplicon sequence variants (ASVs) were taxonomically classified against the SILVA SSU database release 138 (Quast et al., 2013) at 100% identity threshold using VSEARCH (Rognes et al., 2016), and nonbacterial reads were removed. Differences in sequencing depth were corrected using scaling with ranked subsampling (SRS) (Beule & Karlovsky, 2020). The SRS curves were plotted using the 'SRScurve' function, and the ASV table was normalized to 4,798 reads per sample using the 'SRS' function of the 'SRS' R-package (Beule & Karlovsky, 2020).

2.5 | Statistical analysis

Alpha diversity was assessed by determining the Shannon diversity index H' , and species richness determined using the 'diversity' and 'specnumber' function in the 'vegan' R package v2.5-6 (Oksanen, 2017). Differences in exponentially transformed Shannon diversity index H' values and species richness among N treatments were tested using one-way ANOVA with Tukey's honestly significant difference test for separating means. Treatment was considered as fixed effect, and block was considered as random effect. Nonmetric, multidimensional scaling of Bray-Curtis dissimilarities was performed by using the 'metaMDS' function in the 'vegan' R package (Oksanen, 2017). Canonical analysis of principal coordinates (Anderson & Willis, 2003) of Bray-Curtis dissimilarities was performed using the 'CAPdiscrim' function in the 'BiodiversityR' R package v2.11-3 (Kindt & Coe, 2005).

Differences in community dispersion within treatments were examined using a test for homogeneity of multivariate dispersions (PERMDISP) (Anderson, 2006) using 9,999 permutations. PERMDISP was performed using the 'betadisper' function in the 'vegan' R package (Oksanen, 2017). If a homogenous dispersion was given, dissimilarities of soil bacterial community composition in response to N management were tested using nonparametric permutational multivariate ANOVA (Anderson, 2001) using 9,999 permutations. The permutational multivariate ANOVA was performed using the 'adonis2' function in the 'vegan' R package (Oksanen, 2017). Differences in soil bacterial genera abundance were tested using Kruskal-Wallis test with the multiple comparison extension. Statistical significance was considered at $\alpha \leq .05$, and parameters with $p > .05 \leq .08$ are mentioned as marginally significant. All statistical analyses were carried out in R environment v4.0.0 (R Core Team, 2017).

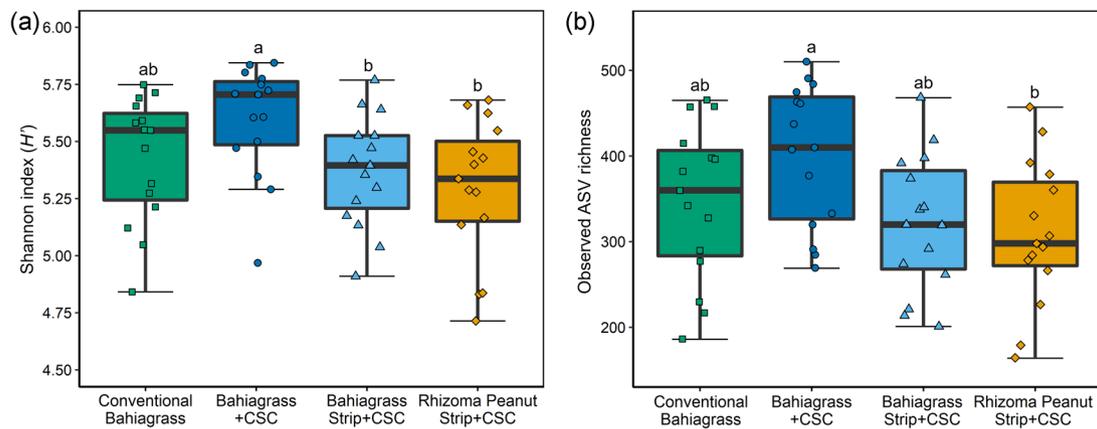


FIGURE 2 Alpha diversity indices of soil bacterial communities across different N management treatments. Boxplot displaying (a) amplicon sequence variant (ASV) diversity (Shannon index H') and (b) ASV richness. Squares, circles, triangles, and rhombi represent individual data points. Different lowercase letters indicate differences in the alpha diversity of soil bacteria (one-way ANOVA with Tukey's honestly significant difference test at $\alpha \leq .05$). CSC, cool-season clover

3 | RESULTS AND DISCUSSION

3.1 | Soil bacterial community composition

Quality-filtered reads from all samples were grouped into 1,069 unique ASVs assigned to 12 phyla. Among all samples, dominant ($>0.5\%$) phyla included Proteobacteria ($24.8 \pm 4.5\%$), Acidobacteria ($23.0 \pm 7.0\%$), Actinobacteria ($15.0 \pm 5.1\%$), Firmicutes ($8.5 \pm 5.6\%$), Verrucomicrobia ($6.0 \pm 2.0\%$), and Bacteroidetes ($5.5 \pm 2.4\%$). Unassigned and rare ($<0.5\%$) phyla accounted for $2.6 \pm 1.1\%$ of all sequences. Phyla representing $<5\%$ relative abundance included Gemmatimonadetes, Myxococcota, Nitrospirae, and Plantomycetes. Bacteria classes Acidobacteriae (Acidobacteria, $17.9 \pm 7.7\%$), Alphaproteobacteria (Proteobacteria, $14.1 \pm 3.1\%$), and Gammaproteobacteria (Proteobacteria, $10.8 \pm 2.7\%$) dominated the samples (Supplemental Figure S1). Approximately 64% of total sequences were assigned at the genus level. We classified ASVs at 100% identity threshold, which likely reduced the number of sequences classified to the genus level but improved taxonomic classification certainty. Among the 27 genera of bacteria observed, the most common across samples included *Bacillus* (Firmicutes, $6.8 \pm 4.6\%$), *Bryobacter* (Acidobacteria, $2.7 \pm 1.0\%$), *Candidatus Udaeobacter* (Verrucomicrobia, $2.5 \pm 1.0\%$), and *Bradyrhizobium* (Proteobacteria, $2.3 \pm 0.8\%$).

3.2 | Soil bacteria community diversity

Soil bacterial diversity (Shannon index H' ; $P = .010$) and richness (observed ASV richness; $P = .022$) were affected by pasture treatments (Figure 2). There was greater bacterial alpha diversity in the Bahiagrass+CSC

treatment than in the Bahiagrass Strip+CSC treatment ($P = .049$) or the Rhizoma Peanut Strip+CSC treatment ($P = .008$) (Figure 2a). In comparison, ASV richness in the Bahiagrass+CSC treatment was greater than in the Rhizoma Peanut Strip+CSC, whereas other treatments were comparable to both ($P = .022$) (Figure 2b). In this case, increasing vegetation diversity did not translate into greater soil bacterial diversity. Successful characterization of the relationship between pasture vegetation diversity and soil microbial diversity remains elusive despite targeted efforts using a range of methods (Kowalchuk et al., 2002; Leff et al., 2015; Millard & Singh, 2010; Prober et al., 2015; Schlatter et al., 2015; Wardle, 2006).

Soil bacteria community composition differed most between the Conventional Bahiagrass treatment and Rhizoma Peanut Strip+CSC based on Bray–Curtis dissimilarities ($P = .025$) (Figure 3). More specifically, pairwise comparisons identified marginal differences between the Conventional Bahiagrass and Rhizoma Peanut Strip+CSC treatments ($P = .076$), as illustrated by nonmetric, multi-dimensional scaling clustering (Figure 3a). The canonical analysis of principal coordinate analysis also showed differences among treatments ($P < .001$) (Figure 3b). In contrast to the Conventional Bahiagrass treatment without legumes, the Rhizoma Peanut Strip+CSC treatment included legumes throughout the year (Garcia-Jimenez, 2019). Previous reports also have demonstrated that legume inclusion affected soil bacterial communities (Grayston et al., 2001; van Eekeren et al., 2009; Zhou et al., 2017). Additionally, Millard and Singh (2010) indicated that the quality of organic matter inputs to soil from different vegetation types may be more important in shaping soil bacterial communities than the plant species themselves. Our results suggest that differentiation between soil bacterial communities in conventional grass pastures versus mixed grass–legume pastures was most likely

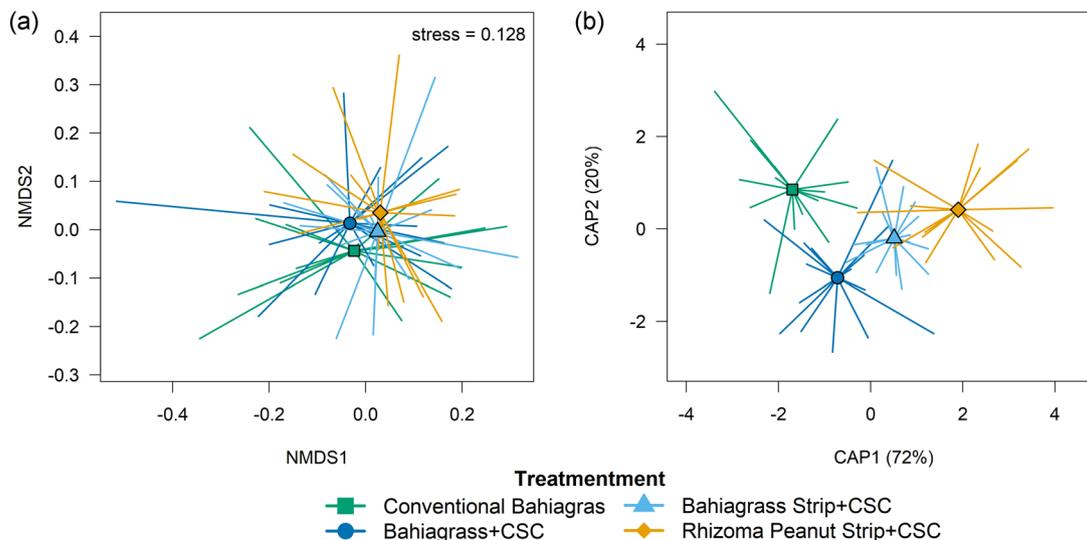


FIGURE 3 Beta diversity among soil bacterial communities under different N management. Nonmetric multidimensional scaling (NMDS) plot of Bray–Curtis dissimilarities calculated for (a) amplicon sequence variants and (b) canonical analysis of principal coordinates (CAP). CSC, cool-season clover

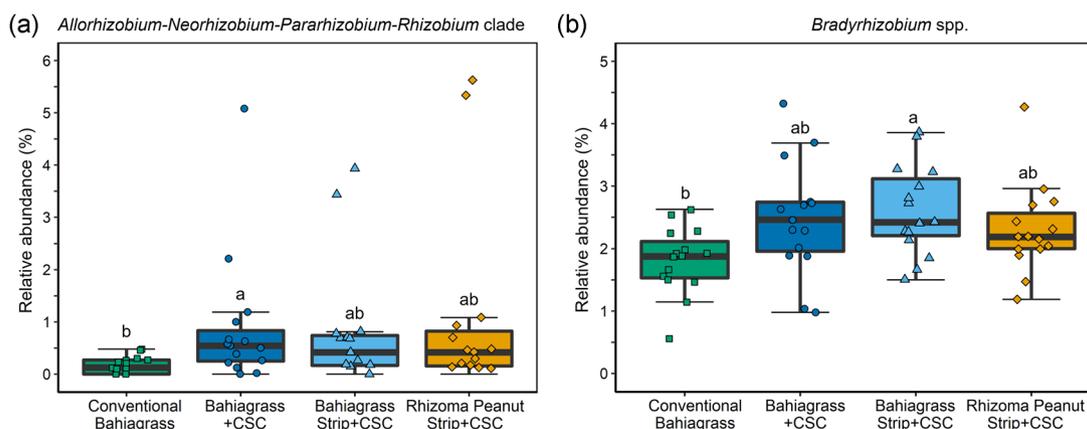


FIGURE 4 Relative abundances of sequences assigned to (a) *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* clade and (b) *Bradyrhizobium* spp. observed in soil samples taken in pastures under different N management treatments. Squares, circles, triangles, and rhombi represent individual data points. Different lowercase letters indicate differences among treatments (Kruskal–Wallis test with multiple comparison extension at $\alpha \leq .05$). CSC, cool-season clover

when there was year-round legume inclusion (i.e., Rhizoma Peanut Strip+CS).

3.3 | Soil bacteria genera relative abundance characteristics

The relative abundances of specific soil bacterial genera known to be involved in N-cycling differed across some treatments (Figure 4). For example, sequences assigned to the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* clade (Proteobacteria) were found in greater abundance in

the Bahiagras+CS than in the Conventional Bahiagras treatment ($P = .024$) (Figure 4a), whereas the Bahiagras Strip+CS had a greater abundance of *Bradyrhizobium* spp. than the Conventional Bahiagras treatment ($P = .012$) (Figure 4b). The genus *Bradyrhizobium* is among the most ubiquitous N_2 -fixing bacteria genera in soil (Nelson et al., 2016), although some species have also been identified as denitrifiers (Bedmar et al., 2005; Ishii et al., 2011). Additionally, some *Bradyrhizobium* spp. are known to persist in soil years after host plant cultivation has terminated (Crozat et al., 1982; Obaton et al., 2002). Even so, shifts in relative abundance of *Bradyrhizobium* among

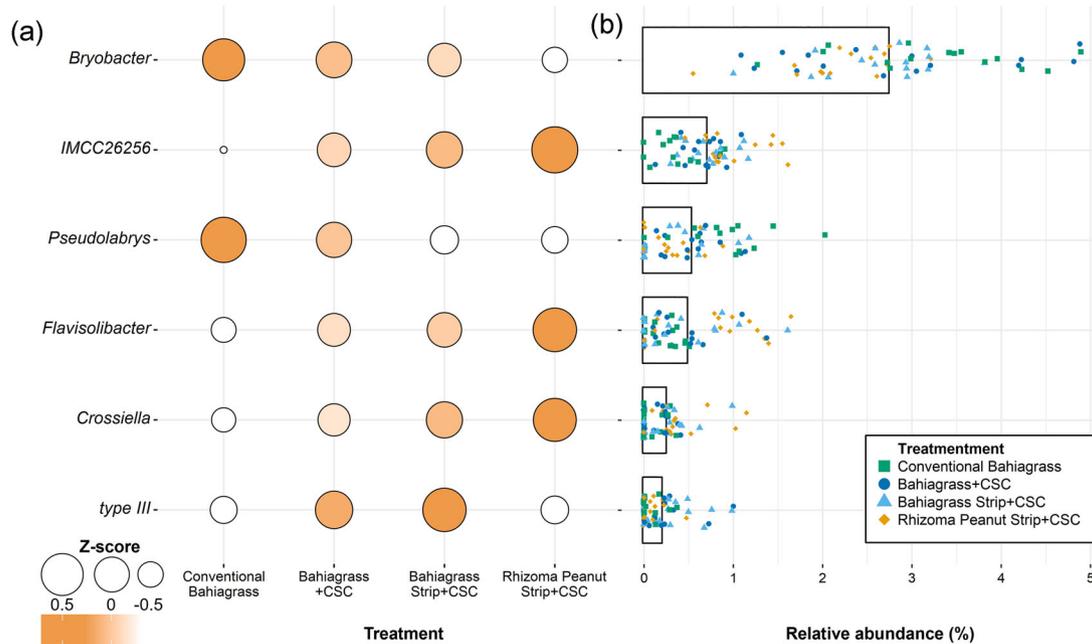


FIGURE 5 Z-score-transformed (a) mean relative abundances and (b) relative abundances of soil bacterial genera identified as having differences among pastures under different N management strategies (Kruskal–Wallis test with multiple comparison extension at $\alpha \leq .05$). CSC, cool-season clover

treatments demonstrate the influential role legume inclusion plays in shaping the soil bacterial community in pastures. Wang et al. (2019) listed *Bradyrhizobium* among the most dominant genera observed in plots across seven different rhizoma peanut cultivars. However, at a nearby site, Beule et al. (2019) reported *Bradyrhizobium* to be the second most abundant genus present among different bahiagrass cultivars.

There was greater relative abundance of *Crossiella* (Actinobacteria) in the Rhizoma Peanut Strip+CSC than in the Conventional Bahiagrass treatment ($P = .017$) (Figure 5a). Sequences assigned to the bacterial genus *type III* (Firmicutes) were found in greater abundance in the Bahiagrass Strip+CSC than in the Rhizoma Peanut Strip+CSC, which lie adjacent to one another, or Conventional Bahiagrass treatments ($P = .010$). Sequences assigned to *type III*, *Crossiella*, and *Flavisolibacter* represented rare taxa (<0.5% relative abundance) whose relative abundances were affected by treatment (Figure 5b).

Flavisolibacter (Bacteroidetes) ($P = .012$) was less abundant in the Conventional Bahiagrass treatment compared with the Rhizoma Peanut Strip+CSC treatment, which was represented by legume species year-round. Additionally, the Bahiagrass Strip+CSC and Rhizoma Peanut Strip+CSC treatments resulted in a greater abundance of uncultured *Actinobacteria* IMCC26256 spp. ($P = .0001$).

There was greater abundance of *Bryobacter* (Acidobacteria) ($P = .032$) in the Conventional Bahiagrass compared with the Rhizoma Peanut Strip+CSC treatment (Figure 5a).

Dedysh et al. (2017) reported that the genus *Bryobacter* preferred substrates composed of sugars, polysaccharides, and organic acids, whereas Rosier et al. (2021) reported a greater abundance of *Bryobacter* in soils at forest edges, which were also positively correlated with soil organic matter. *Bryobacter* spp. were among the most abundant identified soil bacteria across all treatments ($2.7 \pm 1.0\%$) (Figure 5b) and have been suggested to be a keystone taxon among different vegetation types (Xue et al., 2017).

Similar to *Bryobacter*, sequences assigned to the genus *Pseudolabrys* (Proteobacteria) were in greater abundance in the Conventional Bahiagrass treatment compared with the Rhizoma Peanut Strip+CSC but also greater compared with the Bahiagrass Strip+CSC ($P = .009$) (Figure 5a). *Pseudolabrys* was reported to be positively associated with management practices that increase soil organic carbon (Guo et al., 2016; Ho Joa et al., 2014). Patterns of *Bryobacter* and *Pseudolabrys* abundance in this study coincide with Jaramillo, Dubeux, Sollenberger, Mackowiak, et al. (2021), who reported that Conventional Bahiagrass also had the greatest litter deposition among treatments at this site.

Other research has identified positive correlations between the abundance of *Bryobacter* and soil phosphorous availability (Liang et al., 2020; Malviya et al., 2021; Rosier et al., 2021). *Bryobacter*, along with other dominant soil genera in this study, likely contribute significantly to soil nutrient and carbon cycling processes. However, their contributions to soil processes under different land-use and pasture management

practices are far from being fully identified and substantiated. Linkages between soil bacteria community structure and pasture management that affect herbage and root litter, root exudates, and livestock excreta inputs to the soil require further study.

4 | CONCLUSIONS

Changes to soil bacterial community composition under bahiagrass pasture were identified in the southeastern United States in response to different methods of pasture N management. Legume inclusion demonstrated a potential for increasing the proportion soil bacteria capable of performing BNF by up to 44%, whereas mineral N fertilization tended to be affiliated with other soil bacteria, such as *Bryobacter* and *Pseudolabrys* spp., that are associated with more abundant C substrates and nutrient cycling. Increased plant species diversity in pastures did not correlate with increased soil bacterial diversity. However, treatments that contrasted most in their botanical composition also differed in soil bacteria community structure (i.e., relative abundance). Furthermore, our study used next-generation sequencing technology to identify with high taxonomic resolution soil bacterial communities associated with conventional pasture systems found in North Florida. This information contributes to our understanding of how pasture management influences soil bacterial communities, particularly members that are associated with soil C and nutrient cycling. As next generation sequencing becomes a more accessible tool for researchers and as reference taxonomy databases are expanded, investigations into bacterial community diversity and associations between specific taxa and substrate quality and quantity in pasture systems should continue.

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AUTHOR CONTRIBUTIONS

Victor A. Guerra: Formal analysis; Visualization; Writing-original draft; Writing-review & editing. Lukas Beule: Formal analysis; Visualization; Writing-original draft; Writing-review & editing. Hui-Ling Liao: Conceptualization;

Funding acquisition; Methodology; Project administration; Resources; Writing-review & editing. Jose C.B. Dubeux: Funding acquisition; Project administration; Writing-review & editing. Ann R.S. Blount: Writing-review & editing. Xiao-Bo Wang: Writing-review & editing. Diane L. Rowland: Writing-review & editing. Xiao-bo Wang: Writing-review & editing. Cheryl L. Mackowiak: Funding acquisition; Supervision; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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