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Original article

Short-term perennial peanut integration into bahiagrass system influence on soil microbial-mediated nitrogen cycling activities and microbial co-occurrence networks

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ABSTRACT

Integration of perennial peanuts into warm-season grasslands offers a potential solution to reduce nitrogen (N) fertilizer input and enhance N cycling through soil microbial activities. There is limited information on the changes in soil microbial diversity and communities following the short-term integration of rhizoma perennial peanut (RPP; *Arachis glabrata* Benth.) into warm-season perennial bahiagrass (*Paspalum notatum* Flüggé) as well as its impact on N cycling processes. This study investigated changes in N cycling populations and soil microbial communities in bahiagrass-RPP mixtures compared to their monocultures at <2 years after RPP establishment in Spring (March) and Fall (October) seasons. Real-time qPCR was used to quantity N functional groups in the soil involved in nitrification, denitrification, and N_2 fixation. DNA amplicon sequencing was employed to examine co-occurrence networks of soil microbes, while activities of soil enzymes [N-Acetyl-β-d-glucosaminidase (NAG) and leucine aminopeptidase (LAP)] involved in N mineralization were also measured. Bahiagrass-RPP mixtures had no effect on N cycling genes. Ammonia oxidizing archaea were the major ammonia oxidizing prokaryotes compared to ammonia oxidizing bacteria in bahiagrass-RPP systems. We found that bahiagrass-RPP mixtures exhibited greater prokaryotic alpha diversity and NAG activities than RPP monoculture. Meanwhile, RPP influenced soil fungal community composition (beta diversity) and enhanced the relative abundance of dominant soil fungal genera (*Fusarium*, *Gibberella*, and *Humicola*). The presence of RPP in bahiagrass systems led to increased negative microbial interactions in microbial occurrence networks. Greater complexities in microbial networks were linked to forage growth season, which was related to enrichment of the relative abundance of Basidiomycota. Our findings showed that RPP has the potential to influence N cycling process in bahiagrass system by altering the abundance of certain N cycling microbes, especially fungal taxa, within 2 years of RPP establishment.

1. Introduction

Nitrogen (N) is an important nutrient for forage production [1,2]. Its transformation from one form to another and availability to plants are crucial for grassland productivity and sustainability [1,2]. In extensively managed grasslands, where N availability is mainly dependent on microbial-mediated N cycling activities, N limitation is a major factor responsible for decline of grassland productivity [3,4]. Intensively managed grasslands, on the other hand, are faced with negative environmental challenges due to excessive use of inorganic fertilizers or animal wastes [5,6]. Incorporating annual or perennial legumes into grasslands helps maintain productivity while lessening the N fertilizer requirement and environmental concerns [7–9].

Legumes supply N through biological nitrogen fixation (BNF), and they can alter soil chemical properties by releasing N-rich root exudates [10,11]. As a result, soil microbial communities are also altered, in part, by enhancing soil microbial mediated N cycling processes [12,13]. Legumes have been reported to enhance the relative abundance of soil BNF

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bacteria [14,15] but also promote the activities of denitrifying microbial populations [16,17]. Abalos et al. [17] showed that the presence of *Trifolium repens* when combined with grasses (*L. perenne* and *Festuca arundinacea* Schreb) increased N_2O emissions by 58 % and were associated with an increase in the abundance of nitrite (*nir*S and *nir*K) and nitrous oxide reductase (*nos*Z1 and *nos*ZII) genes.

Bahiagrass (*Paspalum notatum* Flüggé) is a major warm-season perennial grass grown in Southeastern United States. It persists well under low soil fertility and low management input but achieves maximum productivity with N fertilization [18]. Rhizoma perennial peanut (RPP) (*Arachis glabrata* Benth.), a warm-season perennial legume, can be grown in mixture with bahiagrass as an alternative to mineral N fertilizer to increase bahiagrass nutritive value and animal daily weight gain [19,20]. Rhizoma perennial peanut, through its association with BNF bacteria, was reported to contribute 30–200 kg atmospheric N ha^{-1} yr^{-1} as either pure stands or as bahiagrass-RPP mixtures [8,9,20,21]. A decomposition study demonstrated that faster decomposition and greater N concentration led to larger litter N release in bahiagrass-RPP mixture than in monocultures [22]. This shows the potential of RPP to promote N cycling processes and increase N availability in bahiagrass pastures.

However, the impact of RPP on N cycling processes may be dependent on the cultivar used. There exist several RPP cultivars, but Florigraze and Ecoturf RPP have undergone more extensive grazing trials. Florigraze was the first cultivar to be released. It has an intermediate growth habit, and it is mainly used as a forage. Ecoturf, on the other hand, is a newly introduced cultivar with a decumbent growth habit and used as forage and for ornamental purposes [20,21]. Dubeux et al. [21] reported that Ecoturf RPP had twice the amount of root-rhizome dry matter and N pool of Florigraze RPP, suggesting that these cultivars might influence below-ground N availability and soil microbial community differently.

Soil microbes play important roles in N cycling and the incorporation of RPP into bahiagrass has been shown to enhance the relative abundance of soil microbial communities such as *Bradyrhizobium* and *Fusarium* that contribute to BNF, N mineralization, and denitrification [15,23]. Nevertheless, we are far from completing a picture of how RPP integrated into bahiagrass affects N cycling microbial populations involved in BNF, nitrification, and denitrification. The abundance of genes encoding key enzymes involved in soil N transformations have been extensively studied and used to evaluate abundances and activity of microbial populations involved in specific N cycling processes [16,24–26]. In addition, soil extracellular enzyme activities, such as N-Acetyl-β-d-glucosaminidase (NAG) and leucine aminopeptidase (LAP) known for degrading chitin and hydrolyzing leucine and other hydrophic amino acids, respectively, have also been measured as proxies for estimating soil N cycling processes [26,27].

To increase our understanding of how perennial legumes impact soil microbial communities and their associated N cycling activities in a perennial warm-season grass system, we investigated N cycling processes, soil microbial diversity and community composition, and microbial co-occurrence networks in a short-term bahiagrass- RPP system (<2 years of RPP integration) compared to their monocultures. The effect of RPP on soil microbial communities have been assessed in bahiagrass-RPP systems following >4 years of RPP integration [15,23], but information on the changes in soil microbial communities and activities within a short period of RPP integration into bahiagrass stand is lacking. Moreover, previous studies on soil microbes in bahiagrass-RPP systems focused on overall soil microbial communities and not those involved in specialized processes like N cycling activities [15,23]. Identifying microbial communities involved in N cycling activities within a short time of RPP establishment into bahiagrass can give a clear picture of temporal progression of soil microbial communities and N dynamics in bahiagrass-RPP systems.

In this study, we quantified N functional groups, using qPCR, targeting genes involved in BNF (*nif*H), nitrification (*amo*A), and denitrification (*nir*K, *nir*S, and *nor*B) in bahiagrass-RPP mixtures and their monocultures at <2 years after RPP establishment in Spring (March) and Fall (October) seasons. We also measured soil extracellular enzyme activities (NAG and LAP), applied amplicon sequencing targeting prokaryotes and fungi, and examined microbial co-occurrence networks. Microbial co-occurrence networks can highlight ecological linkages and give insight to functional and ecological niche occupied by microbial communities [28,29]. We hypothesized that RPP inclusion into bahiagrass stands will increase soil microbial diversity and microbial network complexity as well as increase N cycling genes and microbial taxa responsible for BNF and N mineralization. Compared to Florigraze, Ecoturf RPP will have a greater impact on soil microbial diversity and N cycling genes. Identifying the roles of soil microorganisms in soil N cycling in legume based warm-season grasslands advances our understanding of forage-soil-microbial interactions that can lead to efficient N cycling processes.

2. Materials and methods

2.1. Experimental site and treatment design

The experiment was conducted at North Florida Research and Education Center, Marianna Florida (30° 52′N, 85° 11′W). The soil at the experimental site is classified as Red Bay soil series (fine loamy kaolinitic thermic Rhodic Kandiudults) [30]. Average soil fertility from soil samples collected across each block prior to initiation of experiment was measured as follows: pH (2:1 water: soil) = 5.2, soil organic matter = 2.5 g kg^{-1}, CEC = 5.7 $cmol^{+}$ kg^{-1}, Mehlich-3 extractable P, K, and Mg of 73, 44, and 45 mg kg^{-1}. Weather data from January to December 2020 are presented in Figs. S1A–C, with a total rainfall of 1532 mm compared to the 30-yr average normal of 1298 mm. This study was part of a larger, randomized complete block design with split plots having three different bahiagrass cultivars as whole plots and three RPP cultivars as subplots replicated four times. Only one bahiagrass cultivar 'Argentine' and two RPP cultivars (Ecoturf and Florigraze) were selected for this study. Treatments included 'Argentine bahiagrass (Bahia) and two RPP cultivars [Ecoturf (Eco) and Florigraze (Flo)] and their mixtures (Bahia-Eco and Bahia-Flo). Each plot was 1.5 × 6 m, with four blocks. There were 1.8-m alleys between blocks and no alley between treatments within a block, except for a 3.7 m lane between the second and third plot of each block.

2.1.1. Plot preparation, planting, and plot management

The bahiagrass main plots were already established in 2009, while the RPP treatments were established in May through July 2019. Prior to RPP establishment, from 2012 to 2019, the bahiagrass main plots had been maintained through rotary mowing to approximately 15 cm stubble height, two times each growing season. No other maintenance had been performed. Prior to transplanting RPP into the system, glyphosate [N-(phosphonomethyl) glycine] was applied on 09 May and 16 May 2019 at 11.7 L ha^{-1} (4.8 kg a.i. ha^{-1}) to plots where bahiagrass needed to be removed to ensure successful establishment of RPP as described by Castillo et al. [31]. Additionally, four 30 cm wide strips were sprayed to clear planting areas for RPP in the bahiagrass-RPP mixture plots. The sprayed sections allowed for quicker RPP establishment. Alleys were also maintained using glyphosate applications through a shielded sprayer. All experimental plots were mowed (clippings removed) on 06 June 2019 (7.5 cm stubble height). A manual sod cutter was used to remove 30 cm wide, RPP transplant material from nearby (on-center) production fields. The source fields were at least 5 years. Sod strips were split into 15 cm wide ribbons and planted by hand, into their respective monoculture and mixed plots. For the monoculture plots, the strips were planted into two rows along the length of each respective plot. Florigraze was planted 14 June 2019 and Ecoturf was planted 08 July 2019. Due to abnormally dry conditions in June 2019, a water tanker was used to apply water to new Florigraze transplants on 17, 21, and 27 June

2019. Otherwise, rainfall was the sole source of water. To prevent weed and bahiagrass pressure on RPP, Imazapic and Clethodim [1.17 L ha^{-1} (0.28 kg a.1. ha^{-1}) and 0.29 L (0.07 kg a.1. ha^{-1}) ha^{-1}, respectively] were spot-sprayed over all RPP herbage on 23 August 2019. No herbicide was applied to bahiagrass herbage. All plots received the same fertilizer rate at 22.4 kg N ha^{-1}, 34 kg P_2O_5 ha^{-1}, and 67 kg K_2O ha^{-1}. Fertilizer was applied mid-July in 2019, early May each succeeding year and within a day after the first clipping of each year. No fertilizer was applied after the second clipping each season. The first experimental clipping was 20 June 2020, and second clipping was 15 Oct 2020.

2.2. Soil sampling and analysis

In March and October 2020, five cores were taken each from two ends of the plots at a soil depth of 15 cm using a 2-cm diameter handheld probe and composited, making a total of 80 samples (2 soil cores x 5 treatments x 4 blocks x 2 sampling dates). The samples were stored at −20 °C and transported to the lab 2 to 3 h after collection. Soil samples were passed through a 2 mm sieve to remove roots, debris, and rocks. A subset of the samples was placed in a 2 ml Eppendorf tube, frozen in liquid N_2 for 2 min, and stored at −80 °C for DNA extraction and enzyme analysis [32] The remaining samples were composited per plot for soil chemical analysis. These samples were air-dried and sent to the Soil, Plant, and Water Laboratory, University of Georgia for soil pH, total carbon (TC), and total nitrogen (TN) analysis as described by Erhunmwunse et al. [23]. Subset samples were used to calculate soil water content by drying the subsamples at 105 °C for 48 h.

2.3. DNA extraction and PCR library generation for DNA amplicon sequencing

DNeasy PowerSoil extraction kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from soil samples following manufacturer's instructions. The DNA concentration and quality were checked with NanoDrop™ One (Thermo Scientific, USA) using the A260/280 nm and A260/230 nm absorbance ratios. Primer pairs, 341F/806R [33] and ITS1F/ITS4 [34], were used to amplify the bacterial 16S V3-V4 regions and the fungal internal transcribed spacer (ITS1/ITS2) regions, respectively. Amplicon libraries were prepared using two PCR amplification steps described by Chen et al. [35] in a Labnet MultiGene Optimax Thermal Cycler (Labnet International Inc., USA). At the second PCR stage, barcode tags unique to individual samples were affixed to the tail of the reverse primer. PCR products from the first and second steps were purified using AMPure XP beads (Beckman Coulter, Inc., Indianapolis, IN, USA) and visualized on 1 % agarose gel. Amplicon libraries generated from 80 samples were pooled at 20 ng/μl and sent to Duke Center for Genomic and Computational Biology (GCB) for Illumina MiSeq sequencing (v3 300 bp, 13 Gb).

2.4. Quantitative PCR analysis and soil enzyme assay

Nitrogen cycling-related genes involved in nitrification (*amoA*), denitrification (*nirK*, *nirS*, and *norB*), and nitrogen fixation (*nifH*) were quantified from the extracted DNA samples using a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Primers and PCR cycling conditions as described in the literature were used with minor modification (Table S1). Amplification was performed in 10 μl mixture using 5 μl of PowerUp™ SYBR™ Green Master Mix (Applied biosystems, Lithuania), 0.5 μl of forward and reverse primers (10 μM) specified for each gene, 1 μl of DNA template, and 3 μl of UltraPure DNAse/RNAase free distilled water (Invitrogen, CA). All qPCR runs were carried out in triplicates, followed by melt curve analysis to verify the amplification specificity of genes. Target genes were calculated from the standard curve generated using synthetic standard that contained all primer sequences from Integrated DNA Technologies as gBlocks® gene fragments. The oligonucleotide for each gene of interest was resuspended in UltraPure DNAse/RNAase free distilled water (Invitrogen, CA) and the copy number of each gene in the stock solution was calculated per manufacturer's instructions based on the concentration of the gBlocks gene fragment and the molecular weight. All standard curves showed PCR efficiencies ranging from 80 to 99 % and R^2 values greater than 0.98 (Table S1).

The activities of β-1,4-*N*-acetyl-glucosaminidase (NAG) and L-Leucine aminopeptidase (LAP) were measured using fluorometric method described by Saiya-Cork et al. [36]. Briefly, 2 g of soil was mixed with 125 mL of 50 mM sodium acetate buffer (pH 5.0) and blended for 1 min using Waring Commercial Blender 7011S (Torrington, Connecticut). Aliquots (200 μl each) of soil suspension were added into black 96-well microplates (Greiner Bio-One FLUOTRAC™ 200 96-Well Non-Treated Microplate) that contained 50 μl of 2000 μM of 4-MUB--N-acetyl-B-D-glucosaminide and 100 μM of L-Leucine-7-amido-methylcoumarin hydrochloride fluorescent substrates for NAG and LAP, respectively. Microplates were incubated at 25 °C for 1 h (NAG and 24 h (LAP). After incubation, 10 μl of 1 M NaOH was added to each well to stop the reaction. The fluorescence readings were measured using a Synergy H1 microplate reader (BioTek, Winooski, VT) at 365 nm excitation and 450 nm emissions. The standard curves were determined by mixing 25 μM 4-methylumbelliferone (MUB) for NAG and 250 μM 7-Amino-4-methylcoumarin (AMC) for LAP with soil suspensions corresponding to 0, 0.07, 0.16, 0.31, 0.63, 1.25, 2.5, or 5.0 μM MUB standard solutions and 0, 0.78, 1.56, 3.13, 6.25, 12.5, 25, or 50 μM AMC standard solutions. The enzyme activity was calculated as nmol g^{-1} dry soil h^{-1}.

2.5. Bioinformatic analysis of DNA amplicon sequences and statistical analysis

The prokaryotic 16S rRNA gene and fungal ITS sequences were processed in QIIME 2 [37]. Primers were trimmed using cutadapt version 3.4 in QIIME 2 version 2021.4 [36]. Quality filtering and removal of chimeric sequences were achieved using DADA2. The sequences were processed and assigned to amplicon sequence variant (ASV) at 100 % similarity. Bacterial and fungal ASVs were taxonomically assigned using Greengenes (version 13.8) and UNITE (version 8.3) databases, respectively. Singletons, unassigned, mitochondria, and chloroplast were removed. Rarefaction analyses (Fig. S2) revealed that the sequencing depths (14,000 sequencing depth for prokaryotes and 20,000 sequencing depth for fungi) were sufficient for determining the impacts of forage treatment and sampling date on soil microbial diversity.

Community richness and diversity were determined in QIIME 2 platform using observed features and Shannon index, respectively. Observed features measure the total number of ASVs, while Shannon index considers the total number of ASVs (richness) and their relative abundance (evenness) [38]. Alpha diversity indices, enzyme activities, functional genes, and relative abundance of microbial taxa were analyzed using linear mixed model (with negative binomial distribution) in PROC GLIMMIX in SAS (SAS/STAT 15.1, SAS Institute, Cary, NC). Forage treatments and blocks were considered fixed and random effects, respectively, with sampling date as a repeated measure. The best model for the covariance structure was selected based on the information criterion (AICC). Pairwise comparisons were carried out using Tukey's HSD test. Priori contrasts were also performed to test the effect of treatment groups. The contrasts were as follows: (i) bahiagrass vs RPP; (ii) bahiagrass vs bahiagrass-RPP; and (iii) RPP vs bahiagrass-RPP. Bahiagrass includes bahiagrass monoculture, RPP includes Ecoturf and Florigraze cultivars, and bahiagrass-RPP includes all bahiagrass-RPP mixtures. In addition to graphical observations, Shapiro-Wilk's test was used to assess the normal distribution of the residuals of our variables and Bartlett's tests were used to determine the equality of variance. Beta diversity was estimated using the Bray-Curtis dissimilarity-based principal coordinates analysis with the "vegdist"

function in the vegan package (2.6–4 version). The effect of forage treatment, sampling date, and their interactions on beta diversity was tested using permutational multivariate analysis of variance (PERMANOVA) with "Adonis" function in the *vegan* package. Pairwise comparisons were made for significant main effect of forage treatment using ADONIS. A linear discriminant analysis (LDA) effect size (LEfSe) (using the Kruskal-Wallis sum-rank test) was applied to detect specific microbial taxa, setting a threshold LDA score at 2.0 and *P* value of 0.05 [39]. The LefSE analysis was performed on Huttenhower lab Galaxy server.

2.6. Network analysis

Sequencing data from each forage treatment and sampling date were used separately for network analysis. The ASVs table of the different treatments generated from QIIME 2 were used to construct co-occurrence associations among prokaryotic and fungal communities using the co-occurrence network (CoNet) inference in Cytoscape [40]. Two correlation measures (Spearman and Pearson) and two dissimilarity measures (Kullback–Leibler and Bray–Curtis) were applied to identify pairwise associations [40]. We also performed 1000 renormalization permutation step and bootstraps to minimize potential false-positive correlations and compositionality bias. The *P* values ($P < 0.05$) were merged using the Brown method and adjusted using Bonferroni for multiple testing correction. The network was visualized on the Gephi platform (version 0.9.6) using Fruchterman Reingold algorithms [41]. Network properties were calculated using the statistics tool in Gephi, including the average degree (number of edges connected to a node), network diameter (largest distance between two nodes in a network), average path length (average length of edges within a network), graph density (closeness of a network), modularity (network structure), average clustering coefficient (connectedness among nodes within a network), and percentage of negative correlations (proportion of negative correlations in all correlations of a network) [42]. Positive and negative interactions represent the positive and negative edges which can be used to infer cooperation and competition among microbial nodes, respectively [29].

3. Results

3.1. Soil microbial diversity

Prokaryotic and fungal alpha diversity indices, including Shannon diversity and observed features, were not affected by forage treatment or forage treatment and sampling date interactions (Table 1). However, contrasts among treatment groups showed that prokaryotic alpha diversity in bahia and bahia-RPP mixtures was greater than in RPP monoculture (Table 1). Fungal alpha diversity was impacted by sampling date ($P < 0.001$, Table 1), with greater fungal alpha diversity in October than in March. PERMANOVA analysis showed that prokaryotic and fungal community compositions in March were different from those in October ($P < 0.001$, Table S2; Fig. 1). Fungal communities in RPP plots were different from those associated with bahia or bahia-RPP mixtures ($P < 0.001$, Table S2; Fig. 1B).

3.2. Relative abundance of dominant bacterial and fungal taxa

A total of 36 prokaryotic phyla (99 % total qualified reads) were obtained from the 80 soil samples collected in this study and 456 genera were assigned to 30 % of the total reads. As a result, we focused on the 135 orders assigned to 87 % reads for this study. The predominant bacteria phyla (average ± SE) included Proteobacteria (31 ± 0.4 %), Actinobacteria (22 ± 0.6 %), Acidobacteria (19 ± 0.7 %), Planctomycetes (7 ± 0.2 %), and Chloroflexi (6 ± 0.3 %) (Fig. 2A). Less than 1 % of the total reads assigned to archaea were classified as Crenarchaeota (0.1 ± 0.02 %), Euryarchaeota (0.01 ± 0.002 %), and Parvarchaeota (0.001 ± 0.005 %). A total of 15 phyla (87 % reads assigned) and 431 genera (63 % reads) were identified for fungi. The dominant fungal phyla included Ascomycota (68 ± 1.8 %), Basidiomycota (6 ± 0.7 %), Rozellomycota (6 ± 0.8 %), Mortierellomycota (5 ± 0.7 %), and Glomeromycota (2 ± 0.2 %) (Fig. 2B).

Actinomycetales (13.7 ± 0.42 %), Rhizobiales (10.5 ± 0.33 %), Acidobacteriales (8.2 ± 0.57 %), Rhodospirillales (5.6 ± 0.18 %), and Gemmatales (5.0 ± 0.24 %) were among the major prokaryotic orders (Table 2). The relative abundances of prokaryotic orders were strongly influenced by sampling dates ($P < 0.05$). Apart from the relative abundances of Acidobacteriales and Solibacterales, the relative abundances of bacterial orders were generally greater in March than October (Fig. 3A). Contrasts revealed that in March, the relative abundance of

Table 1
ANOVA results (*P* value) showing the effect of forage treatment (FT), sampling dates (SD), and their interactions on soil extracellular enzymes (NAG and LAP), N cycling genes, and soil microbial alpha diversity indices.

	Source of Variation	[a] NAG	LAP	*amoA* (AOA)	*amoA* (AOB)	*nirK*	*nirS*	*norB*	*nifH*	Prokaryotes		Fungi	
										Observed features	Shannon index	Observed features	Shannon index
	FT	0.158	0.254	0.218	0.078	0.780	0.595	0.482	0.768	0.655	0.889	0.599	0.870
	SD	0.341	0.774	0.061	0.215	0.156	0.010*	0.106	0.992	0.707	0.209	<0.001***	<0.001***
	FT * SD	0.079	0.962	0.100	0.824	0.481	0.319	0.188	0.448	0.086	0.067	0.732	0.986
March													
Contrast 1	Bahia vs RPP	19.3	236.3	5.3	2.2	6.7	3.2	5.3	4.6	124.1	6.3	202.9	5.3
		13.9	257.6	4.7	2.1	6.5	3.0	5.5	4.7	128.8	6.4	204.1	5.1
Contrast 2	Bahia vs Bahia – RPP	19.3	236.3	5.3	2.2	6.7	3.2	5.3	4.6	124.0	6.3	202.9	5.3
		22.3	265.3	4.9	2.5	6.6	2.8	5.4	4.6	122.2	6.3	207.2	5.4
Contrast 3	RPP Bahia-RPP	**13.9b**[††]	257.6	4.7	2.1	6.5	3.0	5.5	4.7	128.8	6.4	204.1	5.1
		22.3a	265.3	4.9	2.5	6.6	2.8	5.4	4.6	122.1	6.3	207.2	5.4
October													
Contrast 1	Bahia vs RPP	15.7	249.8	4.6	2.3	6.6	3.1	5.1	4.6	**131.3b**	**6.3b**	288.6	6.1
		14.0	263.2	4.6	2.3	6.7	3.5	5.0	4.6	**103.4a**	**6.0a**	309.4	6.0
Contrast 2	Bahia vs Bahia - RPP	15.7	249.8	4.6	2.3	6.6	3.1	5.1	4.6	131.3	6.3	288.6	6.1
		19.2	271.5	4.8	2.7	6.8	3.4	5.4	4.8	135.1	6.3	334.9	6.2
Contrast 3	RPP vs Bahia-RPP	**14.0b**	263.2	4.6	2.3	6.7	3.5	5.0	4.6	**103.4b**	**6.0b**	309.4	6.0
		19.2a	271.5	4.8	2.7	6.8	3.4	5.4	4.8	**135.1a**	**6.3a**	334.9	6.2

*** and * indicate significance at $P < 0.001$ and 0.05. [††]Different letters indicate a significant difference among contrasts, according to *F* test at $P < 0.05$.

[a] N-Acetyl-β-d-glucosaminidase (NAG); leucine aminopeptidase (LAP); ammonia monooxygenase gene (*amoA*), ammonia oxidizing archaea (AOA) and bacteria (AOB); nitrite reductase genes (*nirK* and *nirS*); nitric oxide reductase gene (*norB*); nitrogenase gene (*nifH*).

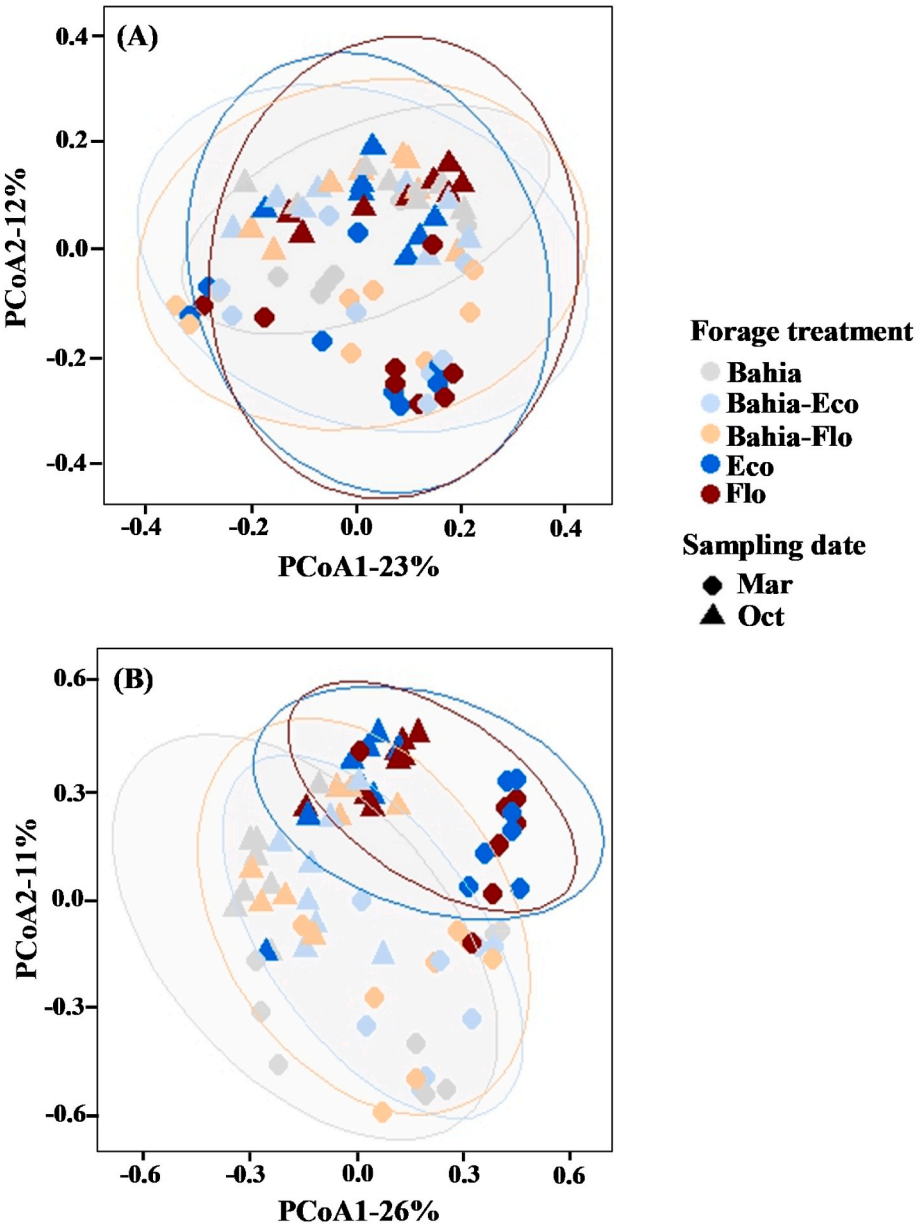

Fig. 1. Principal component analysis based on Bray-Curtis dissimilarity (using ASV tables) showing the effect of forage treatments and sampling dates on (A) prokaryotic and (B) fungal community compositions. Multilevel pairwise comparison (Adonis) for fungal communities (Table S2) showed significant differences between bahiagrass-based treatments (Bahia = Bahia-Eco = Bahia-Flo) ≠ and RP monocultures (Eco = Flo) at $P = 0.005$.

Acidobacteriales was greater in bahia than in bahia-RPP mixtures and RPP plots, while in October, Acidobacteriales were greater in RPP than in bahia and bahia-RPP mixtures (Table S3). In March, Bacillales in bahia and RPP monocultures were greater than in bahia-RPP mixtures.

Mortierella (5.3 ± 0.78 %), *Fusarium* (5.0 ± 0.59 %), *Humicola* (4.4 ± 0.66 %), *Trichoderma* (4.4 ± 0.81 %), and *Penicillium* (3.6 ± 0.50 %) were among the major fungal genera detected in the collected soil (Table 2). Unlike prokaryotic groups, the relative abundances of dominant fungal genera were mainly affected by forage treatment and forage treatment and sampling dates interactions ($P < 0.05$, Table 2). Fungal genera *Epicoccum*, *Humicola*, *Mortierella*, and *Trichoderma* had greater relative abundance in March than October (Fig. 3B), following the pattern that was found for the largest fungal phylum, Ascomycota, as well as Mortierellomycota (both occupy 74 % of the entire fungal community). Contrasts showed that the relative abundance of *Clonostachys*, *Fusarium*, and *Gibberella* in RPP monoculture were greater than in bahiagrass associated treatments across March and October and the same pattern was observed for Humicola only in March (Table S3). The relative abundance of *Epicoccum* and *Paraphaeosphaeria* were greater in bahia/bahia-RPP mixtures than RPP (Table S3).

Linear discriminate analysis (LDA) effect size (LEfSe) was applied to identify differentiating microbial taxa among forage treatments and between sampling dates. The results showed that there were no differential prokaryotic taxa among forage treatments. At prokaryotic order, eleven taxa were enriched in October and six taxa in March (Fig. 4B). Bacterial orders belonging to Actinobacteria (Actinomycetales) and Firmicutes (Bacillales) were distinct in March and taxa belonging to Acidobacteria (Solibacterales and Acidobacteriales) were prevalent in October. Meanwhile, fungal genera such as *Clonostachys*, *Fusarium*, and *Gibberella* were enriched in Eco and Flo plots. *Nothophoma* and *Epicoccum* were prevalent in the mixtures, while *Alfaria* and three other fungal genera were enriched in bahia (Fig. 4A). At fungal genera, fifteen fungal taxa were differentiated in March and seven in October (Fig. 4C). Fungal genera belonging to Ascomycota and Mortierellomycota were

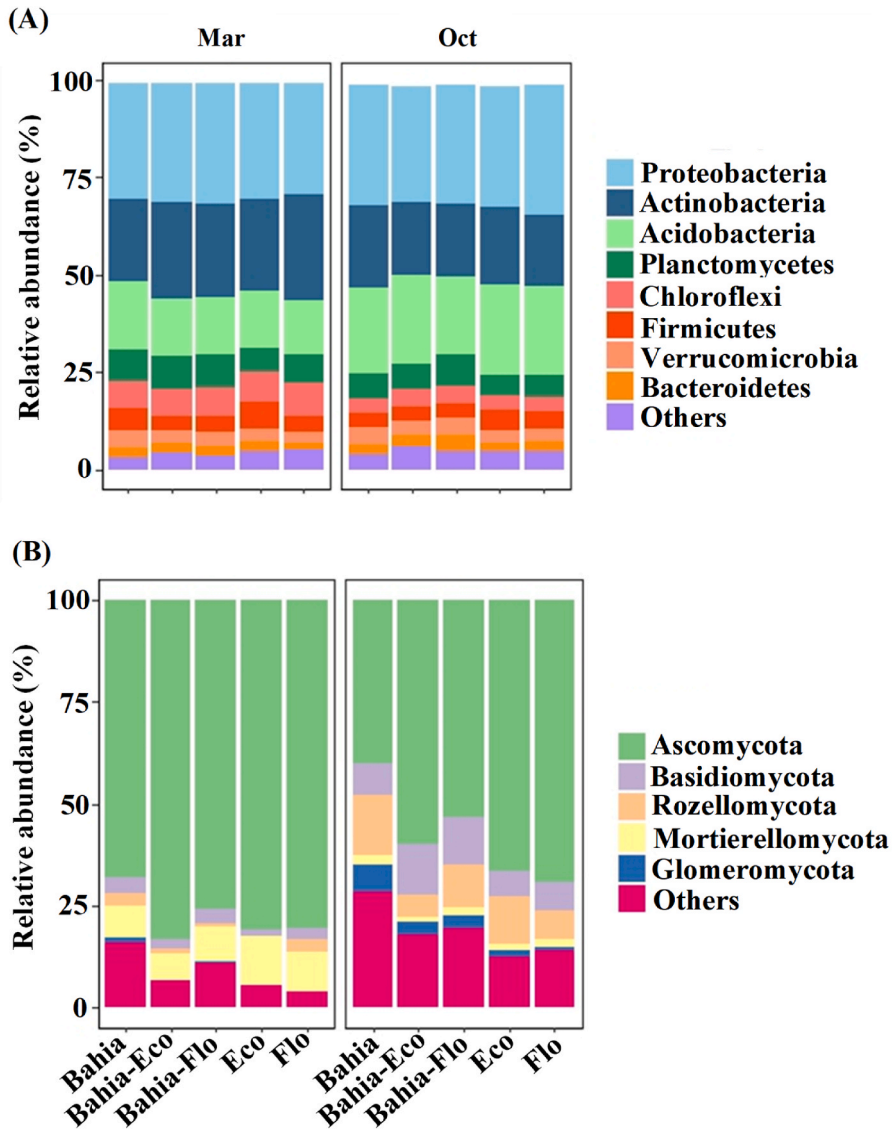

Fig. 2. Relative abundance of dominant (>1 %) (A) prokaryotes and (B) fungi at phylum level among forage treatments at the two sampling dates.

mainly prevalent in March and members of Basidiomycota and Glomeromycota were enriched in October.

3.3. Soil prokaryotes and fungal association networks

Co-occurrence networks were constructed to illustrate soil prokaryotic and fungal networks for each forage treatment and sampling date (Figs. S4 and S5). For prokaryotic networks, the highest number of nodes (63) and edges (80), as well as co-occurrence properties, such as network diameter and average path length, were found in Bahia-Eco. The lowest number of nodes (30) and edges (20) were observed in Flo (Table 3). However, we found more negative interactions among prokaryotic networks in Bahia-Flo (36.2 %) and Flo (35 %) compared to the other treatments, with negative interactions of 1.4–5.2 %. The top 10 most abundant prokaryotes (Table 2) were among the keystone orders in prokaryotic networks, with Rhizobiales and Actinomycetales occupying 23 % and 16 %, respectively, of the networks across forage treatments (Fig. S4A). Prokaryotic networks between sampling dates showed that there were greater numbers of nodes, edges, and negative interactions in October (node, 190; edge, 459; and neg, 38.1 %) than in March (node: 117, edge: 192, and neg: 31.8 %) (Table 3). Some prokaryotic orders, such Caulobacterales and Gemmatimonadales. were only found in March, while Crenarchaeales, Pedosphaerales, and Planctomycetales were detected in October (Fig. S4B).

Similar to prokaryotic network results, the highest number of fungal network nodes (41) and edges (56), as well as cooccurrence patterns, such as network diameter and average path length, were found in Bahia-Eco. In comparison, the fewest numbers of nodes (32) and edges (28) were observed in Bahia-Flo (Table 3). *Fusarium* was the predominant genus identified in the fungal networks across forage treatments and sampling dates (Fig. S5A). The fungal genus, *Trechispora*, was identified only in bahiagrass based treatment (Bahia, Bahia-Eco, and Bahia-Flo). *Acremonium* and *Stagonosporopsis* were present in RPP monocultures (Eco and Flo), while *Alfaria* and *Umbilicaria* were only present in the bahiagrass monoculture. Fungal network analysis by sampling date showed a greater number of nodes (74) and edges (78) in October than in March (nodes, 66) and (edges, 73). Additionally, more negative interactions were observed in March (42.5 %) than October (23.1 %). The fungal genera *Alfaria*, *Dictyosporium*, and *Xenopenidiella*, were detected in March, while *Epicoccum*, and *Wongia* were detected in October (Fig. S5B).

Table 2
ANOVA results (*P* value) showing the effect of forage treatments (FT), sampling dates (SD), and their interactions on the relative abundance of top ten bacterial and archaeal orders and fungal genera. The predicted ecological functions of these taxa were also listed.

Taxa	Rel abun (%)	FT	SD	FT x SD	Ecological function[1]		References
Bacterial orders					Ecotypes	Mediated paths[2]	
Acidobacteriales	8.2	0.472	<0.001***	0.002	Epiphytes/Endophytes/ decomposers/ Saprotrophs	Anammox, C degradation, N metabolism (Nitrate and nitrite reduction	[56]
Actinomycetales	13.7	0.410	<0.001***	0.073	Decomposers/ Epiphytes	N-cycling and denitrification	[55,102]
Bacillales	4.4	<0.001***	0.004**	0.013*	Epiphytes/ Endophytes/ Decomposers/	C, N-cycling, and denitrification	[103]
Burkholderiales	3.0	0.492	<0.001***	0.912	Diazotrophs	N fixation, denitrification	[55]
Gaiellales	2.9	0.129	0.016*	0.452	Denitrifiers	Nitrate reduction	[104]
Gemmatales	5.0	0.248	<.0001	0.382	Decomposers	C and N cycling	[57,105]
Nitrospirales	0.3	0.585	0.540	0.700	Nitrifiers	Nitrification	[57,96]
Nitrososphaerales	0.03	0.499	0.019	0.633	Nitrifiers	Nitrification	[57]
Rhodospirillales	5.6	0.056	0.091	0.103	Diazotroph	C and N fixation	[106]
Rhizobiales	10.5	0.434	0.171	0.166	Symbiont	N-fixation and denitrification	[96,107,108, 109]
Solibacterales	5.0	0.475	0.006**	0.080	Decomposers/ Saprotroph	Denitrification and anammox	[96]
Solirubrobacterales	3.6	0.069	0.059	0.912	Decomposers/ Saprotroph	Org N metabolism	[95]
Fungal genera							
Clonostachys	2.2	<0.001***	0.255	0.009**	Pathotroph/ Saprotroph/ Symbiotroph	C and N cycling	[110,111]
Epicoccum	2.6	<0.001***	<0.001***	0.032*	Pathotroph/ Saprotroph/ Symbiotroph	C and N cycling	[112]
Fusarium	5.0	<0.001***	0.735	0.530	Pathotroph/ Saprotroph/ Symbiotroph	C and N cycling; Denitrification	[113,114]
Gibberella	1.5	<0.001***	0.281	0.056	Pathotroph/ Saprotroph/ Symbiotroph	C and N cycling; Denitrification	[113,114]
Humicola	4.4	0.118	0.002**	0.049*	Pathotroph/ Saprotroph	C and N cycling	[115]
Mortierella	5.3	0.214	<0.001***	0.346	Saprotroph/ Symbiotroph	C and N cycling	[113,116]
Nothophoma	2.8	<0.001***	0.253	0.004**	Pathotroph/ Endophytic/ Saprotoph	C and N cycling	[117]
Paraphaeosphaeria	0.9	<0.001***	0.304	0.004**	Saprotroph	C and N cycling	[113]
Penicillium	3.6	0.470	0.051	0.062	Saprotroph	C and N cycling	[113,118]
Trichoderma	4.4	0.267	0.002**	0.173	Pathotroph/ Saprotroph/ Symbiotroph	C and N cycling	[113,119]

[1] The combination of literature reviews and FUNGuild for fungi were applied to predict the major ecological function of each taxon, and fungal genera. ***, **, and * indicate significance at $P < 0.001$, 0.01, and 0.05.

3.4. Nitrogen cycling genes and enzyme activities

Forage treatment had no significant effect ($P > 0.05$) on soil extracellular enzyme activities (NAG and LAP) and N cycling genes *amo*A (AOA and AOB), *nirK*, *nirS*, *norB*, and *nifH* (Table 1). However, based on contrasts among treatment groups, NAG activity in bahiagrass-RPP mixtures was greater than in RPP monoculture in March (Table 1). There was a significant effect of sampling date on *nirS* ($P = 0.013$) and a marginal effect ($P = 0.061$) was observed for AOA (Table 1). The gene copy number for *nirS* was greater in October than March. Meanwhile, the gene copy number of AOA was greater in March than October (Table 1). Among the ammonia oxidizing groups, AOA populations (~ log 5 gene copies/g soil) were the dominant groups than AOB (~log 2 gene copies/g soil). Similarly, *nirK* (~ log 7 gene copies/g soil) were more abundant than *nirS* (~ log 3 gene copies/g soil) (Table 1).

4. Discussion

4.1. Overall patterns of soil microbial communities and N cycling populations in response to short-term RPP integration into bahiagrass system

The integration of legumes into grassland pastures is considered a sustainable option for maintaining forage productivity, increasing N cycling, and promoting the growth of beneficial soil microorganisms [4,43]. Well-established RPP plots have been shown to enhance soil N cycling activities and promote microbial taxa involved in N cycling in Florida warm-season pastures [9,15,23,44]. However, the short-term integration of RPP on soil microbial communities and N related cycling activities are yet to be studied, despite more than half of herbage production being reported in the first two years of RPP establishment [8,45]. In a low-N input system, legumes in grass-legume mixtures may promote microbial diversity, enzyme activities, and N cycling processes [46,47]. Our findings showed that regardless of cultivars, <2 years of RPP integration into already existing bahiagrass stands impacted

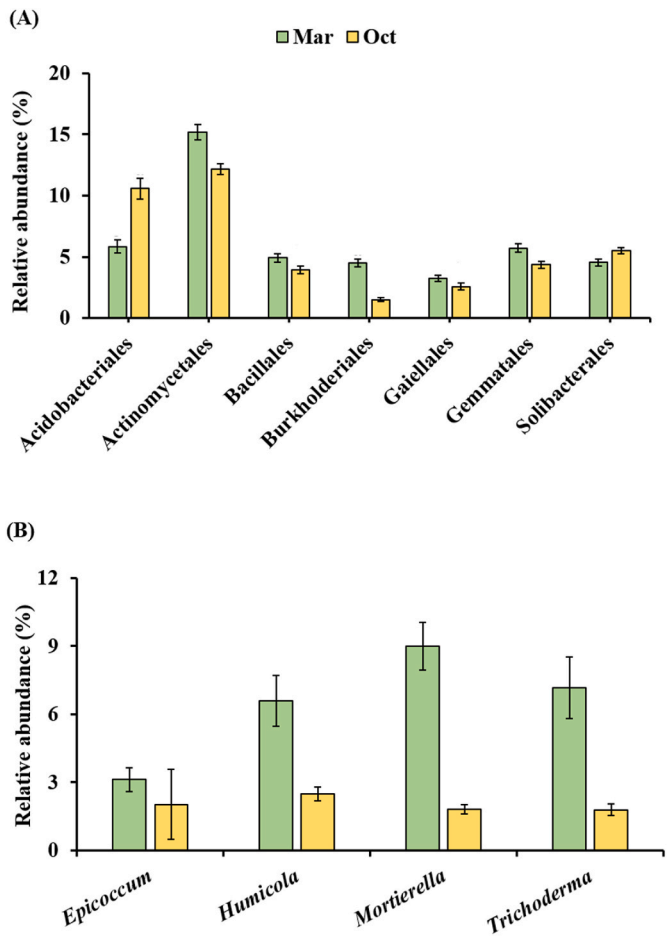

Fig. 3. The relative abundance of the dominant (A) prokaryotic and (B) fungal taxa that showed significant responses to sampling dates according to the ANOVA results on Table 3. Only differential abundant taxa are shown.

enzyme activities and microbial diversity. However, soil prokaryotic and fungal diversities were differentially impacted. Bahiagrass-RPP mixtures resulted in greater NAG activities and prokaryotic alpha diversity than RPP plots (Table 1). Meanwhile, soil prokaryotic beta diversity was not different among forage treatments (Fig. 1A), indicating that the presence of RPP did not lead to changes in soil prokaryotic community compositions in bahiagrass systems. On the other hand, RPP had no influence on fungal alpha diversity, which is indicative of the abundance and evenness of the soil fungal taxa (Table 1). However, soil fungal community compositions in RPP plots were distinctive from those in bahiagrass monoculture and bahiagrass-RPP mixtures (Fig. 1B).

Changes in prokaryotic diversity and community composition have been demonstrated to be minimally impacted by plant host communities and mainly explained by changes in soil properties such as soil moisture, temperature, and physicochemical properties [10,48,49]. The measured soil chemical properties (soil pH, TC, and TN; Table S4) in RPP and bahiagrass-RPP plots in our study were similar and could not explain the lower soil prokaryotic alpha diversity and NAG activities in RPP compared to bahiagrass-RPP plots. It is noteworthy to mention that herbicides were applied for the killing of bahiagrass plants in the RPP plots as opposed to no herbicides used in bahiagrass plots and a little portion applied in bahiagrass-RP mixtures. Herbicides have been shown to have negative impact on soil bacterial communities [50] and might have played a role in the reduced prokaryotic diversity and NAG activities observed in RPP plots.

Unlike the higher variability and fast evolving ITS region which enables us to conduct taxonomy identification of fungal communities at the genus level (Table 2) [51,52], the 16S regions is conserved for prokaryotic communities coupled with the short reads from next generation sequencing. As a result, we conducted prokaryotic taxonomic classification at the order level in this study (Table 2) [53,54]. Most of the dominant prokaryotic orders (Actinomycetales, Bacillales, Burkholderiales, Nitrospirales, and Rhizobiales) identified in bahiagrass-RPP systems contain species primarily involved in C and N cycling, especially anammox, nitrate/nitrite reduction, N-fixation, denitrification, and C degradation (Table 2) [55–57]. They were also reported in other southeast US forage systems [49,58–60]. The lack of influence of forage treatments on prokaryotic beta diversity extended to individual prokaryotic taxa at order level. Prokaryotic community composition and individual taxa were mainly influenced by sampling dates. Temporal shifts of soil microbial communities in agricultural systems are widely reported and attributed to changes in climate conditions, plant growth stages, and substrate availability [61,62]. From our study, soil pH and total carbon were the major drivers of changes in prokaryotic communities (Table S4; Table S5). This is consistent with previous studies [23,49]. Soil pH may indirectly affect soil prokaryotic communities by influencing soil nutrient availability, such as micronutrients, calcium, magnesium, and toxic ions (Al^{3+}) [63].

In contrast to soil bacterial diversity, plant host communities have been reported to affect soil fungal diversity [64,65] and this might be due to the plethora of plant hosts associated with diverse fungal groups [64–66]. In line with previous findings, fungal alpha and beta diversities were positively correlated with aboveground dry matter and plant nutrient concentration (Table S5). Greater fungal alpha diversity in October than in March is likely to be influenced by the temporal changes in the quantity and quality of plant litter [9,67] and linked to increased relative abundance of fungal phyla, Basidiomycota, Glomeromycota, and Rozellomycota in October (Fig. 2B). Compared to October, warm-season perennial forages in North Florida are beginning to resume growth from their winter dormancy in March. Bahiagrass and RPP are photoperiod sensitive and severely limited in March [68,69]. Furthermore, forages were clipped a day prior to sample collection in October, which might signal increased root exudation and residue deposits that favored diverse fungal groups, such as mycorrhizae, saprotrophs, and decomposers [70,71]. Consistent with fungal beta diversity, the relative abundances of dominant fungal genera were greater in RPP stands than in bahiagrass and bahigrass-RPP mixtures. We observed that these fungal genera, e.g *Fusarium* and *Gibberella* were positively correlated with plant N concentration or C/N ratios (Table S5), indicating that the higher the plant N, the greater the relative abundance of the fungal taxa. This might explain their abundance in RPP plots. Members of these fungal genera have potential roles in N cycling processes. *Fusarium*, *Gibberella*, and *Humicola* enhanced under RPP plots are known for their role in soil organic matter decomposition [72,73], which can contribute to N mineralization and increase plant N availability [74]. Soil fungi also contribute to nitrification and denitrification processes [75,76]. *Fusarium* and *Gibberella* isolated from cattle pasture were found to be potent nitrous oxide producers [76].

In our study, AOA were more abundant than AOB, suggesting that archaea were the major players driving nitrification in bahiagrass-RPP systems in Florida. Previous studies have acknowledged the major role of archaea in nitrification [77,78]. Ammonia oxidizing archaea have been shown to exhibit oligotrophic attributes and they were found to be negatively correlated with N [79]. This could be linked to their abundance in our system, with low-N input. Likewise, *nirK*-harboring denitrifiers were more prevalent in our study than the *nirS* denitrifiers. *NirS* and *nirK* constitute two biologically distinct denitrifying groups that are believed to be mutually exclusive [80]. *NirK* denitrifiers have been reported to be taxonomically diverse than *nirS* denitrifiers [80,81]. Previous studies have shown that *nirS*-denitrifiers were more sensitive to environment changes such as pH, moisture, and C:N ratio than *nirK*--denitrifiers in grasslands and cropping systems [80,82,83]. This might have contributed to the temporal difference in *nirS* genes that we

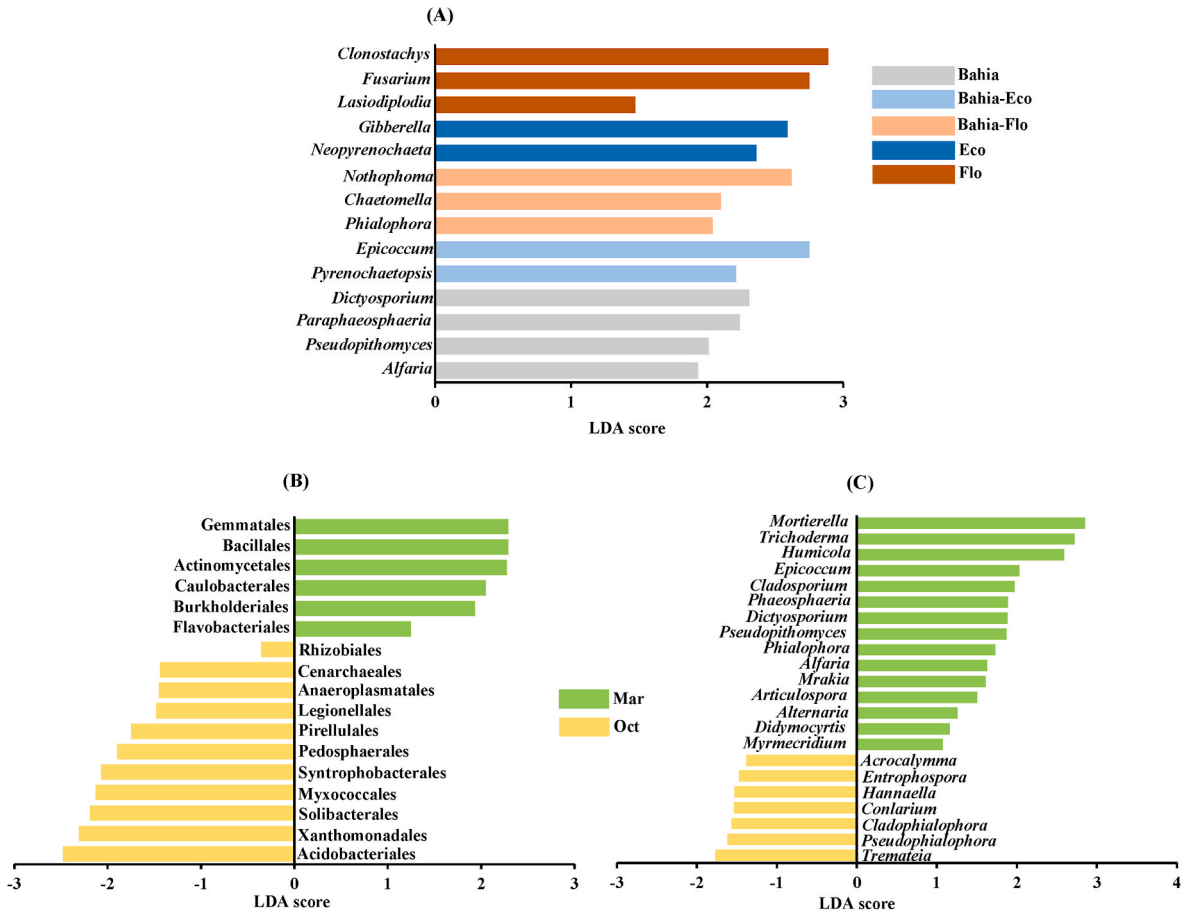

Fig. 4. Taxa explaining differences between experimental factors: A) fungal genera among forage treatments, B) bacterial order between sampling dates, C) fungal genera between sampling dates. Linear discriminant analysis effect size (LEfSe) of microbial communities with LDA scores higher than 2.0 are shown using the bars.

Table 3
Network properties among forage treatments and between sampling dates for prokaryotic orders and fungal genera.

Properties	Forage treatments										Sampling dates			
	Bahia	Bahia-Eco	Bahia-Flo	Eco	Flo	Bahia	Bahia-Eco	Bahia-Flo	Eco	Flo	Mar	Oct	Mar	Oct
	Prokaryotes					Fungi					Prokaryotes		Fungi	
Nodes	57	41	63	52	30	39	41	32	33	38	117	190	66	74
Edges	71	78	80	77	20	56	56	28	40	33	192	459	73	78
Average degree	2.03	3.81	2.42	2.70	0.65	2.87	2.73	1.75	2.42	1.74	1.80	3.46	2.21	2.11
Network diameter	9	8	10	7	4	10	13	6	8	7	9	9	7	7
Average path length	3.43	3.28	4.07	2.70	2.12	4.12	5.12	2.51	2.96	2.91	3.88	3.47	3.17	2.53
Graph Density	0.029	0.095	0.037	0.048	0.011	0.076	0.068	0.056	0.076	0.047	0.009	0.013	0.034	0.029
Modularity	0.828	0.704	0.748	0.729	0.755	0.635	0.787	0.596	0.761	0.592	0.826	0.764	0.811	0.807
Clustering coefficient	0.258	0.272	0.069	0.152	0	0.331	0.238	0.342	0.397	0	0.236	0.073	0.157	0.309
Positive interactions (%)	98.6	87.2	63.8	94.8	65	96.4	98.2	96.4	100	97.0	68.2	61.9	57.5	76.9
Negative interactions (%)	1.41	12.8	36.2	5.2	35	3.6	1.8	3.6	0	3.0	31.8	38.1	42.5	23.1

observed, with a greater copy number in October than March.

Overall, the introduction of RPP into bahiagrass system did not impact N fixing genes measured in our study. Our soil samples were a mixture of rhizosphere and bulk soils and might have masked the impact of RPP on the abundance of N cycling genes in this study. Root exudate is one of the several ways plant species influence soil microbial community, therefore rhizosphere soils and roots analysis may yield a different result [84]. Future studies should also focus on fully profiling the C/N associated genes and enzymes in soil using advanced analysis, such as metagenomics and metaproteomics, to gain substantial information on how RPP influence soil microorganisms in bahiagrass systems and the role of these microorganisms in soil fertility, nutrient cycling, and warm-season forage growth.

4.2. Soil microbial network characteristics in bahiagrass and RPP system

In agricultural soils, interactions within microbial communities, whether positive or negative, are important for community assembly and ecological function. For example, plant litter decomposition involves a succession of microbial communities that depend on substrates produced by other microorganisms [85]. Negative interactions or competition through antibiosis can result in elimination of potential plant pathogens in soils and promote plant growth [86]. In our study, prokaryotic networks in Florigraze RPP based systems (Bahia-Flo and

Flo) showed more negative interactions (indicating competitive exclusion) than in other forage systems. Direct competition for resources and niches, production of toxins, changes to the environment, and niche adaptation can all lead to negative interactions among soil microbes [87]. Varied degrees of negative edges/interaction may indicate different levels of competition and niche specialization among microorganisms [29]. For instance, low levels of negative interaction suggest a higher level of collaboration and niche sharing [88]. It is unclear why there was a higher percentage of negative interactions in Florigraze RPP based treatments compared to others. Rhizobiales and Actinomycetales were not only among the top 10 prokaryotic taxa identified (Table 2), but also prevalent in the networks across forage treatments (Fig. S4A and Table S6). This suggests that members of Actinomycetales and Rhizobiales might play important roles in ecological functioning and nutrient cycling in bahiagrass and RPP systems [89–92].

Microbial networks in October suggested more complex bacterial and fungal communities than in March. Several factors might be responsible for this trend observed. Previous studies have shown that microbial networks are less complex under environmental stress due to reduced microbial diversity [93]. As mentioned earlier, forage growth is limited in March in North Florida due to winter dormancy and episodes of winter frost. We identified some unique bacterial orders at each sampling date. Caulobacterales and Gemmatimonadales were detected only in March. Caulobacterales, a member of Alphaproteobacteria, has been reported to play an active role in litter decomposition [94] and these microbes have been shown to be oligotrophic. For example, relative abundance of Caulobacterales was greater under low N soil [95], this might explain their presence in March when soil N was lower (Table S4). Most of the prokaryotic orders including Cenarchaeales, Pedosphaerales, and Planctomycetales identified solely in October (Fig. S4B and Table S6) are closely related to N cycling. Members of Crenarchaeales and Planctomycetales have been reported in ammonia oxidation and assimilatory nitrate reduction, respectively, in grassland soils [96].

Fusarium is likely a predominant fungal group in grasslands soils in North America [23,97]. *Fusarium* was identified as a primary fungal genus in all fungal networks across forage treatments and sampling dates, as it was highly connected with other fungal genera. This supports findings by Erhunmwunse et al., [23], where *Fusarium* was identified as a dominant fungal genus across bahiagrass and RPP pastures in Florida. *Fusarium* play various roles in grassland soils. While some members contribute to litter decomposition and nutrient cycling through their saprophytic lifestyle, others are important plant pathogens [98,99]. More negative interactions among fungal nodes in March compared to October suggest that competition within fungal community intensified in March (Table 3). A plausible explanation may be less plant growth in March resulting in substrate limitation and stimulating direct competition within fungal communities. Interestingly, most fungal genera belonging to Basidiomycota were predominant in the fungal networks in October. This was in line with greater relative abundance of Basidiomycota, Glomeromycota, and Rozellomycota across forage systems in October indicating that these fungal phyla may be used as biomarkers for specific seasons in bahiagrass and RPP systems. Basidiomycota are saprobic and mainly responsible for plant litter and wood decomposition. Forage quality tends to be higher in spring than in late season, and this may be reflected in plant litter composition [100]. This may explain a succession from Ascomycota in March to Basidiomycota in October as Basidiomycota produce enzymes to degrade complex organic materials [101].

5. Conclusion

This study showed that short-term incorporation of rhizoma perennial peanut into bahiagrass system differentially impacted prokaryotic and fungal alpha and beta diversities. The inclusion of rhizoma perennial peanut into bahiagrass resulted in greater prokaryotic alpha diversity and changes in soil fungal community compositions. We found that rhizoma perennial peanut promoted the relative abundances of soil fungal genera such as *Gibberella*, *Fusarium*, and *Humicola* known for their roles in soil organic matter decomposition, N mineralization, and nitrification. Furthermore, ammonia oxidizing archaea and n*ir*K-harboring denitrifiers were more abundant than ammonia oxidizing bacteria and *nir*S-harboring denitrifiers, suggesting that they are the key players of nitrification and denitrification in bahiagrass-rhizoma perennial peanut systems.

Bacterial orders, Actinomycetales and Rhizobiales, as well as fungal genus, *Fusarium,* were the keystone microbial taxa in microbial co-occurrence network, an indication that they might play important roles in ecological functioning and nutrient cycling in bahiagrass and rhizoma perennial peanut systems. Sampling time also affected microbial networks as greater complexities in microbial networks were greater in October linked to plant growth season compared to March. Most fungal genera belonging to Basidiomycota were prevalent in microbial networks in the month of October.

Overall, this study showed that within a short time of establishment in bahiagrass systems, rhizoma perennial peanut has the potential to influence N cycling activities by increasing soil prokaryotic diversity and increasing fungal mediated N-cycling processes involving organic material decomposition and N mineralization.

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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.

Data availability

The data have been deposited in a public repository

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2023.103566.

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