Use of cover crops and grazing management on row cropland: BMPs to reduce ground water nitrates

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Executive Summary</td>
<td>3</td>
</tr>
<tr>
<td>2. Background</td>
<td>3</td>
</tr>
<tr>
<td>3. Justification</td>
<td>4</td>
</tr>
<tr>
<td>4. Objectives</td>
<td>6</td>
</tr>
<tr>
<td>5. Material and Methods</td>
<td>6</td>
</tr>
<tr>
<td>6. General Results and Discussion</td>
<td>11</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td>i. Grazing intensity affects cover crop responses without affecting cotton lint yield</td>
<td>12</td>
</tr>
<tr>
<td>ii. Above and belowground litter decomposition of cover crops grazed at different intensities</td>
<td>28</td>
</tr>
<tr>
<td>iii. Sward responses of bahiagrass managed across different grazing intensities in a sod-based rotation system</td>
<td>49</td>
</tr>
<tr>
<td>iv. Short-term soil responses to contrasting grazing intensities in integrated crop-livestock systems</td>
<td>66</td>
</tr>
<tr>
<td>v. Nitrate leaching in contrasting cropping systems</td>
<td>83</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
</tr>
<tr>
<td>i. Water footprint, herbage, and livestock responses in N-fertilized and grass-legume grazing systems</td>
<td>89</td>
</tr>
<tr>
<td>ii. Nitrate leaching from grazing systems with contrasting N inputs</td>
<td>108</td>
</tr>
<tr>
<td>7. Products from the project</td>
<td>114</td>
</tr>
<tr>
<td>8. References</td>
<td>122</td>
</tr>
</tbody>
</table>
1. EXECUTIVE SUMMARY

The overall goal of this project was to reduce nitrate leaching from agroecosystems in North Florida, along the Blue Spring Basin and Chipola River watersheds. Two research actions were performed at UF IFAS North Florida Research and Education Center (NFREC) in Marianna, FL. In action 1, we investigated the use of integrated crop-livestock systems as a tool to reduce nitrate leaching and increase adoption of cover crops in North Florida. We addressed a range of treatments including fallow, cover crop without grazing, and grazing cover crops at different intensities in a sod-based rotation system (SBR) or in an integrated crop-livestock system (ICL) without the sod component during the warm season. We had drain lysimeters installed in each plot and measured nitrate leaching. In addition, we measured soil, forage, and crop responses as affected by treatments. In action 2, we investigated the possibility to replace inorganic N fertilizer by introducing forage legumes in grazing systems. We assessed soil, plant, and livestock responses along with nitrate leaching from these systems. We also assessed the water footprint of N fertilized vs. grass-legume mixed systems. Results from action 1 indicated that the use of cover crop reduces nitrate leaching and the winter is the hotspot for nitrate leaching. Grazed plots also resulted in lesser nitrate leaching compared to ungrazed plots. There were no differences between ICL and SBR regarding nitrate leaching and both were better than ungrazed and fallow plots. Cotton lint yield was not affected by grazing. In action 2, we were able to replace 234 kg N ha\(^{-1}\) yr\(^{-1}\) by 34 kg N ha\(^{-1}\) yr\(^{-1}\) when we moved from N fertilized to grass-legume mixed systems, maintaining and/or increasing the livestock performance per area (and per animal). Nitrate leaching was reduced in grass-legume mixtures, and beef produced in grass-legume mixtures had lesser water footprint (i.e., less water required per unit of beef produced). Results from this project must be scaled up in North Florida to increase sustainability of crop and livestock production systems while reducing nitrate load in the environment.

2. BACKGROUND

Pursuant to the Florida Watershed Restoration Act (FWRA), section 403.067(7)(c)3, F.S., the Florida Department of Agriculture and Consumer Services (FDACS), Office of Agricultural Water Policy (OAWP), develops, adopts, and assists with the implementation of agricultural Best Management Practices (BMPs) to protect and conserve water resources. Funding for BMP projects that complement the OAWP’s mission is consistent with FWRA objectives. In this regard, the University of Florida’s, Institute of Food and Agricultural Sciences (UF/IFAS) continues to play an important role in assisting the industry with implementing BMPs. Effective July 1, 2016, new research priorities have emerged per the requirements pursuant to Section 373.813, FS. This three-year project proposes to demonstrate a variety of grazing management (stocking rates), type of cover crop, and nitrogen (N) fertilization rate as a means to improve crop productivity and reduce nitrate leaching in the Jackson Blue Spring/Merritts Mill Pond, and Chipola River watersheds.
3. **JUSTIFICATION**

Jackson County has among the largest concentration of row crop farms in North Florida, which account for 40% of its current land-use. Traditional row crop production requires nitrogen applications, where a single crop of corn may receive as much as 240 lbs/A of N applied during the growing season and the land remaining fallow until the next season. Without cool-season cover crops, residual nitrogen likely will leach below the root-zone before the subsequent cash crop is planted. This poses a potential water quality problem for sensitive areas, such as the Jackson Blue Spring basin. The recently signed Basin Management Action Plan (BMAP) imposes a 0.35 mg/L nitrate-nitrogen (NO₃-N) total maximum daily load (TMDL) on the Jackson Blue Spring/Merritts Mill Pond Basin, as nitrate concentrations have been increasing linearly over the past 50 years (Figure 1). According to the Florida Department of Environmental Protection, the majority of this N originates from agricultural sources. Nitrate concentrations need to decrease 90% to meet the adopted TMDL.

![Figure 1. Nitrate and total nitrogen concentration in Jackson Blue Spring (Source: BMAP Jackson Blue Spring and Merritts Mill Pond).](image)

The use of cool-season cover crops is crucial for capturing residual fertilizer nutrients left behind by warm-season row crops, in order to reduce nitrate leaching. Grazing cover crops is an effective method to recycle nutrients, enhance the succeeding crop root system, and reduce nitrate leaching in integrated crop-livestock systems. However, the effectiveness of grazed cover crop management and crop selection (including crop mixtures) on N leaching losses is unknown. For example, there is no information on the upper limit for stocking pressure on resulting cover crop residues that carry over to the summer crop. This information is important
because crop residue (above- and below-ground) acts like a bridge for conserving nutrients from one summer cropping season to the next.

Grazing intensity will affect cattle gains, cover crop performance and the amount of residues that carry into the summer cropping season. Since a large portion of the Jackson Blue Spring/Merrits Mill Pond BMAP agricultural land is devoid of cover crops during the winter, these data will be necessary for assessing potential economic and environmental benefits of adopting recommended practices. Row crop and livestock producers need scientifically sound guidance that will result in sustainable agricultural gains, along with adequate nitrate conservation. There is no known reported work linking the aforementioned management practices with nitrate leaching.

Successful integration of crops and livestock into a 160-acre sod-based rotation system (SBR) has been in place for over 14 years, at the North Florida Research and Education Center (NFREC), Marianna. The system has been fine-tuned to provide ecosystem services, as well as economic benefits to landowners. Benefits of the SBR include improving crop productivity, reducing irrigation requirements, soil organic matter (SOM) increases, and reducing nitrate leaching. This farming system routinely reduces crop N inputs by nearly 50%, while also reducing irrigation requirements. Currently, the SBR system uses a mixture of cool-season grasses (rye and oat) and N fertilization (100 lbs N/acre) during the cool-season. To reduce N fertilizer inputs further during the cool-season, we propose using a grass/legume mixture that benefits cattle performance. The infrastructure for this grazing trial is in place, including drain and porous cup lysimeters in replicated paddocks. Data from this long-term study will lead to SBR management improvements by reducing potential N leaching during the cool-season and transition periods.

Introducing into the SBR a cool-season grass/legume mixture (instead of 100 lbs N/acre on cool-season grasses) in Jackson County on cotton/peanut rotations on 48,000 acres may reduce applied N by 5.9 million lbs/yr, while also increasing farm income. To help realize this outcome, UF-IFAS investigators propose to demonstrate cattle productivity under a grass/fertilized system and compare it to a grass/clover system. We will also monitor the impact of cattle on the system, measuring nitrate leaching and cattle performance among contrasting systems. An economic analysis will support SBR use by row crop producers. These data support larger SBR efforts, by addressing the limited cattle performance data available from SBR systems.

All data collected during this project, including cover crop type, stocking rate, and N fertilizer input, will be included in SBR BMP developments. Additionally, the information should contribute to the Row Crop and Cow-calf BMP manuals, as well. The ultimate goal is to develop economical and reasonable agricultural practices that help to reduce nitrate leaching in sensitive water basins. The overriding questions that we are trying to answer with this project are:

1. Do cover crops reduce nitrate leaching?
2. Can we graze cover crops and still have the same benefits (reduced nitrate leaching and greater row crop productivity) as ungrazed sites?
3. What is the best stocking rate and residual biomass on cool-season cover crops to minimize nitrate leaching and maximize warm-season row crop productivity?
4. Is the sod-based rotation system better than integrated crop-livestock systems regarding nitrate leaching and row crop performance?
5. Can we replace soluble N fertilizer during the cool-season with forage legumes to further reduce nitrate leaching and still maintain cattle performance in a sod-based rotation system?

4. OBJECTIVES

- Develop best management practices regarding the use of cool-season cover crops and grazing vs. non-grazing, to reduce nitrate leaching and enhance row crop productivity.
- Develop BMPs specifically for grazing cover crops in the SBR system.
- Contrast sod-based rotation (SBR) system with integrated crop-livestock (ICL) systems (similar to SBR but without the warm-season sod in the rotation) to assess nitrate leaching and crop productivity.
- Determine the optimum grazing intensity (stocking rate) to reduce nitrate leaching and enhance crop productivity.
- Generate livestock performance and economic data for the SBR during the cool-season using current management practices (grass + 100 lbs N/acre) and reduced N input (grass/legume mixture + 30 lbs N/acre).
- Demonstrate results obtained in this project to stakeholders, including producers, allied industry, and official agencies. Schedule annual producer meetings.

5. GENERAL MATERIAL AND METHODS

The general approach for the overall project is presented in this section. Detailed approach will be presented subsequently, based on each subject. We are addressing the research objectives by performing the following two experiments at our satellite sites: Experiment 1) assess the effect of cover crop, grazing intensity, and rotation system type on nitrate leaching and row crop productivity, and Experiment 2) assess the effect of reducing N fertilization from 100 lb N/acre to 30 lb N/acre by replacing N fertilizer with legumes and measure treatment impact on nitrate leaching and cattle productivity. In combination, the experiments will generate economic information regarding cattle enterprises on pasture and row-crop land use, while also contributing needed data specifically to SBR systems. Best technologies identified in small-scale plots (Experiment 1) will be tested at a mid-size trial (Experiment 2) where cattle performance
and economic information can be generated from contrasting systems. The best technologies and economic information generated for livestock performance from experiment 2 will be implemented into the 160-acre SBR demonstration site in Marianna, FL in future projects (Figure 2).

![Figure 2. Diagram demonstrating the scaling-up of tested technologies, from small-plots to grazing trials, and then to the demonstration site, the sod-based rotation system located in Marianna (160 acres). Figure not drawn to scale.](image)

**Experiment 1. Grazing intensity on cover crop: How does stocking rate affect nitrate leaching and warm-season crop productivity in sod-based rotation (SBR) versus an integrated crop-livestock (ICL) system?**

**Materials and Methods Experiment 1**
Location: The study is located at the North Florida Research and Education Center (NFREC) in Marianna (30°52’ N, 85°11’ W, 35 m altitude). The soil at the experimental site is comprised of Red Bay fine sandy loam and Orangeburg loamy sand. These soils are representative of the Jackson Blue Spring and Chipola River Basins.

Procedures: We are assessing the effect of cover crop, grazing intensity, and rotation system (SBR or ICL) on nitrate leaching and crop productivity during the warm-season. The SBR rotation uses bahiagrass (YRs 1 and 2) and peanut (Yr 3), while the ICL rotation uses cotton (Yrs 1 and 2) and peanut (Yr 3). The ICL system is an intermediate step between the traditional row-crop...
systems and the SBR. In the ICL, there is the benefit of grazing cover crop and enhancing nutrient cycling during the cool-season, but it does not rely on a warm-season, perennial grass (i.e., bahiagrass) during the warm-season. Row crop farmers would plant all irrigated land with row crops during the warm-season and plant grazing cover crops over the entire area during the cool-season. This system might enhance nutrient cycling and add economic benefit through livestock weight gains. A complete randomized block design will be used to assess treatments (plots), with three replications.

Treatments:
Row crop, no cover crop and no grazing (Control treatment)
Row crop, rye/oat cover crop (30 lbs N/acre) and no grazing (Typical cover crop system)
Row crop, rye/oat cover crop (80 lbs N/acre) and no grazing (cover crop with same rate of grazing trials)

**SBR:** Row crop, rye/oat cover crop + 9 lbs N/acre (grazed every 2 wks. leaving 500 lb. DM/acre of post-grazing residual stubble mass)
**SBR:** Row crop, rye/oat cover crop + 80 lbs N/acre (grazed every 2 wks. leaving 1,500 lb. DM/acre of post-grazing residual stubble mass)
**SBR:** Row crop, rye/oat cover crop + 80 lbs N/acre (grazed every 2 wks. leaving 2,500 lb. DM/acre of post-grazing residual stubble mass)

**ICL:** Row crop, rye/oat cover crop + 80 lbs N/acre (grazed every 2 wks. leaving 500 lb. DM/acre of post-grazing residual stubble mass)
**ICL:** Row crop, rye/oat cover crop + 80 lbs N/acre (grazed every 2 wks. leaving 1,500 lb. DM/acre of post-grazing residual stubble mass)
**ICL:** Row crop, rye/oat cover crop + 80 lbs N/acre (grazed every 2 wks. leaving 2,500 lb. DM/acre of post-grazing residual stubble mass)
Cool-season cover crops were established in the Fall of each year (2017, 2018, and 2019). Cool-season plantings followed the warm-season crop (cotton for years 2018, 2019, and 2020) component of the SBR. Each plot measures 24 x 50 ft. and is individually fenced. After planting, every 2 weeks the cool-season cover crop was evaluated to determine herbage mass and herbage accumulation. Grazing commenced when herbage mass reached 1,000 lbs Dry Matter/Acre and grazing events occurred every 2 weeks thereafter. Plots were only grazed if herbage mass was greater than the post-grazing residual stubble mass. Cattle was closely monitored during grazing and was withdrawn from the paddock when residual stubble mass reached target height. Stubble height was linked to stubble mass using a double-sampling procedure (Haydock and Shaw, 1975). Disk settling heights of an aluminum disk were taken (30 per paddock) every 14 days during each grazing season, before placing the cattle. Settling heights were the indirect measurements. These pairs were calibrated with harvested samples every 28 d. Regression equations were developed for each post-grazing stubble height treatment using 18-paired samples (disk settling height and its respective harvested sample). After developing the equations, the average disk settling height of each paddock were used as the independent variable to estimate herbage mass. Grazing time for each plot was recorded in order to estimate grazing days and stocking rate for each treatment.

Drain lysimeters were placed in three blocks, with one lysimeter per plot (total of 27 drain lysimeters) at 1.5 m (5 ft) depth. Leachate was collected biweekly. Samples were analyzed for NO₃ using the EPA 353.2 method and NH₃ using the EPA 350.1 method. Leachate samples were collected directly into a labeled sterile 20-ml scintillation vials (Fisher catalog 0333723C),
preserved with sulfuric acid (H₂SO₄) to pH < 2, kept in a cool container (~4°C), and analyzed within 28 days from sampling. Leachate samples were sent to a certified UF laboratory in Gainesville, FL.

Crops were managed under irrigation, using a central pivot system. Irrigation management was based on soil moisture and evapotranspiration. Warm-season crop (cotton) was planted approximately from mid-April to mid-May. Crop establishment and management followed UF/IFAS recommendations. Crop production and harvest index were determined by harvesting a strip 3 x 10 ft. within each plot. Because of hurricane Michael in 2018, the cotton crop was lost. We were able to sample the losses and the cotton in the plant and will present the data. Subsamples were collected to determine N concentration to estimate N exported by crops. In both the integrated crop-livestock system and sod-based rotation system, we used conservation tillage to reduce soil erosion and help build up of SOM. Cool-season cover crops were no-till drilled on either bahiagrass sod (SBR) or crop residue (ICL). Rye and oats were planted at 50 lbs/acre each, totaling 100 lbs/acre of seed. Nitrogen was applied three weeks after planting (30 lbs N/acre) and after the first grazing event (50 lbs N/acre) on plots fertilized with 80 lbs N/acre rate. Other nutrients were applied following IFAS recommendations for the specific crop/cover crop. Baseline, surface (0-6” soil layer) samples were collected before initiating the trial for soil organic matter (SOM) and fertility determination. Surface soil samples were collected annually, thereafter. Soil inorganic N (ammonium and nitrate) were measured at 5 depths (0-15, 15-30, 30-60, 60-90, and 90-120 cm), at initiation and end of the trial.

**Experiment 2. Reducing N inputs from fertilizers in the Sod-Based Rotation System during the cool-season: Can we reduce nitrate leaching by replacing N fertilizer with cool-season legumes?**

**Materials and Methods Experiment 2**

**Location:** The study is adjacent to the demonstration SBR system at the North Florida Research and Education Center (NFREC), Marianna (30°52’ N, 85°11’ W, 35 m altitude). The soils at the experimental site are Ultisols, Red Bay fine sandy loam and Orangeburg loamy sand. These soils are representative of the Jackson Blue Spring and Chipola River Basins.

**Procedures:** We are replacing N fertilizer with biological N₂-fixing forage legumes and assessing the effect on nitrate leaching and livestock productivity. Currently, paddocks contain nine drain lysimeters (one per experimental unit; and replicated three times) and 18 porous cup lysimeters (two per experimental unit; and replicated three times). Data obtained during the cool-season can be applied to the SBR winter system. Treatments are assigned in a complete randomized block design, with three replications.

**Grazing system treatments:**

1. Rye/oats + 100 lb. N/acre (similar to SBR) during the cool-season and 100 lbs N/acre during the warm-season on bahiagrass;
2. Rye/Oat/Crimson/Red/Ball clover + 30 lbs N/acre during the cool-season and unfertilized bahiagrass during the warm-season; and

3. Rye/Oat/Crimson/Red/Ball clover + 30 lbs N/acre during the cool-season and unfertilized bahiagrass/perennial peanut during the warm-season.

Cool-season forages were no-till drilled onto dormant bahiagrass sod during the fall. Seeding rates for rye and oat were similar to the ones used in Experiment 1, i.e., 50 lbs of seed/acre of each component (100 lbs/acre of the mix). Clover seeding rate was 15, 6, and 3 lbs/acre for crimson, red, and ball clovers, respectively. In 2019-2020 cool-season, cereal rye was replaced by annual ryegrass cv. ‘Prine’. Nitrogen was applied three weeks after planting (30 lbs N/acre) and after the first grazing event (70 lbs N/acre; 50% as Environmentally Smart Nitrogen and 50% as urea) on plots fertilized with 100 lbs N/acre rate. Other nutrients were applied following IFAS recommendation for the specific crop/cover crop.

Nitrate leaching was monitored with drain and porous cup lysimeters. The data from the drain lysimeters were not useful because problems in the equipment. In June 2019, new drain lysimeters were installed. Nitrate sampling followed a similar protocol described in Experiment 1. Herbage mass and nutritive value were determined following Dubeux et al. (2016). Cattle performance was determined every 21 days after a 16-h fasting period. Cattle average daily gain (ADG) was calculated for each 21-d period by dividing the average weight gain of the two tester (same animals remain in treatments during entire season) animals during that specific period by the number of days. Grazing days were calculated by multiplying the number of livestock in each paddock by the number of days within each period. Adjusted numbers were used to express grazing days per acre. Gain per area estimates were determined by multiplying the average daily gain by the grazing days per acre within each period.

Statistical procedures for both trials
Data was analyzed using PROC MIXED in SAS (SAS for Windows V 9.4; SAS Institute, 2009). For experiment 1, the fixed effects included grazing intensity, rotation system, year, and sampling date. For experiment 2, fixed effects included grazing systems, year, and sampling date. In both trials, blocks were considered random effect. Means were compared using LSMEANS procedure adjusted for Tukey’s test (P ≤ 0.05). The repeated measures procedure was used for repeated measurement variables. Differences were considered significant at P ≤ 0.05.

6. RESULTS AND DISCUSSION

Experiment 1

In this experiment, we addressed the following topics:

i) Grazing intensity affects cover crop responses without affecting cotton lint yield
ii) Above and belowground litter decomposition of cover crops grazed at different intensities

iii) Sward responses of bahiagrass managed across different grazing intensities in a sod-based rotation system

iv) Short-term soil responses to contrasting grazing intensities in integrated crop-livestock systems

v) Nitrate leaching in contrasting cropping systems

Results and summary of each one of the above topics are detailed below.

i) Grazing intensity affects cover crop responses without affecting cotton lint yield

**Summary:** Grazing cover crops can improve land use and diversification, making it more resilient to market fluctuations. We investigated how grazing intensity affects cover crop forage responses and cotton (*Gossypium hirsutum* L.) lint yield. Cover crops were a mixture of rye (*Secale cereale* L.) and oat (*Avena sativa* L.), managed as follows: no cover (control), no grazing + 34 kg N ha⁻¹ (NG34), no grazing + 90 kg N ha⁻¹ (NG90), overgrazing (OG), moderate grazing (MG), and undergrazing (UG). Grazed treatments also received 90 kg N ha⁻¹. Average post-grazing herbage mass (HM) obtained for OG, MG, and UG were 520, 1350, and 2120 kg DM ha⁻¹, respectively. Herbage accumulation (HA) rate was greater earlier in the season, and greater for UG than OG, with MG being intermediate. Crude protein (CP) and in vitro digestible organic matter (IVDOM) decreased along grazing cycles and were usually greater for OG than MG and UG. Stubble residue before cover crop termination was greatest for NG34 and NG90 in 2018 and 2020, however, in 2019 NG90 had greater stubble residue before termination than NG34 (7540 vs. 6650 kg DM ha⁻¹). Overgrazing resulted in greater weed proportion (17 vs. 6.5%) and lesser soil cover (49 vs. 70%) than non-grazed cover crops. Cotton lint yield was not affected by treatment, and it was often low, reaching its maximum in 2019 at 520 kg ha⁻¹. Grazing intensity did not affect cotton lint yield, but these results could be different in the long-term because of soil responses.

**Methods**

**Experimental Site**

The experiment was conducted during three consecutive years, 2018, 2019, and 2020 at University of Florida, Institute of Food and Agricultural Sciences, North Florida Research and Education Center, Marianna, FL (30°52' N, 85°11' W). The soil at the experiment site was a Red Bay fine sandy loam (Fine-loamy, kaolinitic, thermic Rhodic Kandiudults) (USDA Soil Survey Staff, 2020). Weather data during the experiment years are presented in Figure 4. Initial soil samples taken in May 2017 reported pH\textsubscript{water} of 6.1, 7.9 g kg⁻¹ of soil organic matter, and Mehlich-1-extractable P = 12.5 g kg⁻¹, K = 36.5 g kg⁻¹, Mg = 57.5 g kg⁻¹, Ca = 358 g kg⁻¹, S = 10 g kg⁻¹, B = 0.16 g kg⁻¹, Zn = 0.9 g kg⁻¹, Mn = 30.5 g kg⁻¹, Fe = 8 g kg⁻¹, and Cu = 0.25 g kg⁻¹.

**Treatments and Experimental Design**

‘Florida 401’ rye and ‘RAM’ oat were planted using a no-till drill at a rate of 56 kg ha⁻¹ each on 1 Dec. 2017, 20 Nov. 2018, and 25 Nov. 2019 on plots that measured 7.3 x 15.2 m. Treatments were replicated three times in a randomized complete block design and were as follows: no cover crops and no grazing (control), no grazing + 34 kg N ha⁻¹ (NG34), no grazing + 90 kg N ha⁻¹ (NG90), overgrazing (OG), moderate grazing (MG), and undergrazing (UG). All grazed treatments were fertilized with 90 kg N ha⁻¹. The cover crop fertilizer was split-applied at approximately 3-wk after planting and after the first grazing event. Except for the control, all treatments were fertilized with 34 kg N ha⁻¹, 17 kg P ha⁻¹, 74 kg...
K ha⁻¹, and 3.4 kg B ha⁻¹ in the first fertilization. During the second fertilization, except for the control and NG34, all treatments were fertilized with 56 kg N ha⁻¹.

**Grazing Management**

The Institutional Animal Care and Use Committee (IACUC) of the University of Florida approved all procedures for the experiment (protocol #202009924). Plots were grazed every 14 days using Angus heifers (*Bos taurus taurus*) to maintain the target stubble residue treatments of 500 (OG), 1500 (MG), and 2500 kg DM ha⁻¹ (UG). Average body weight (kg) and standard deviation for the experimental animals at the beginning of the grazing season in 2018, 2019, and 2020 were 509 ± 30, 411 ± 39, and 474 ± 32, respectively. The first grazing occurred when the forage mass was 500 kg DM ha⁻¹ greater than the OG target stubble residue (i.e., 1000 kg DM ha⁻¹), which occurred on 15 Feb. 2018, 7 Feb. 2019, and 29 Jan. 2020. Because grazing started later in 2018 than in 2019 and 2020, there were five grazing cycles in 2018, whereas there were six grazing cycles in 2019 and 2020.

The double-sampling technique (Haydock & Shaw, 1975) was used to estimate pre- and post-grazing forage mass. The technique consists of regressing aluminum disk settling heights (indirect measurement) and forage mass harvested in the area (0.25 m²; direct measurement). The equations were calibrated every 28-d using 24 paired samples. Before grazing, disk settling heights (30 points per paddock) were taken to determine pre-grazing HM using the indirect measurement. Disk settling heights were also taken during the grazing event. Once the target height was reached, the animals were removed from the plots and post-grazing HM was recorded.

**Cover Crops Termination and Cotton Management**

Cover crops were terminated on 17 Apr. 2018, 17 Apr. 2019, and 15 Apr. 2020, right after the last grazing event, by applying 4.7 L ha⁻¹ of glyphosate (Buccaneer Plus®; N-(phosphonomethyl)glycine). After termination, cover crops were mowed using a Woods Bush hog, on 7 May 2018, 25 Apr. 2019, and 4 May 2020.

Six rows of ‘Delta Pine 1646’ cotton (dicamba resistant) were planted in each plot on 17 May 2018, 8 May 2019, and 5 May 2020 at 10 seeds per linear meter using a cotton planter (Hayvan no till drill, AG-Meier Industries, LLC, Belton, TX, USA). Weeds were controlled in the paddocks using Engenia® [dicamba: N,N-Bis-(3-aminopropyl)methylamine salt of 3,6- dichloro-o-anisic acid] and Roundup PowerMAX II [Glyphosate, N-(phosphonomethyl)glycine, in the form of its potassium salt] as described in Table 1.

Cotton was fertilized twice each year. On 8 June 2018, 11 June 2019, and 21 May 2020 the plots were fertilized with 17, 22, and 84 kg ha⁻¹ of N, P, and K, respectively. In the second fertilization, only N was applied at a rate of 72 kg ha⁻¹ and it occurred on 25 June 2018, 31 July 2019, and 19 June 2020. Cotton was defoliated on 29 Oct. 2018, 5 Nov 2019, and 1 Oct 2020 by using a mix of Finish® 6 Pro [(2-chloroethyl)phosphonic acid and 1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid] at a rate of 1.75 L ha⁻¹, DEF 6 (S-S-S-Tributyl phosphorotrithioate) at a rate of 0.74 L ha⁻¹, and Dropp SC [Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) at a rate of 0.12 L ha⁻¹.

**Response Variables**

Forage variables included pre- and post-grazing HM, HA rate, standing residue, IVDOM, and CP. Pre- and post-grazing HM were determined as described above, using the double sampling technique (Haydock and Shaw, 1975). The HA rate was determined during two grazing cycles of each year, one during the early season and another during the late season, by the difference of the pre-grazing HM of the current grazing cycle and the post-grazing HM of the previous cycle, divided by the number of days between grazing cycles. The early season in 2018, 2019, and 2020 were from 12 to 26 Feb., 18 Feb. to 4 Mar., and 29 Jan. to 12 Feb, respectively. The dates for the late season measurements on HA rate in 2018, 2019, and 2020 were 12 to 26 Mar., 18 Mar. to 1 Apr., and 25 Mar. to 9 Apr. The standing residue was taken after the last grazing cycle of each year by using disk-settling heights and applying the double-sampling equation in all treatments containing cover crops.
Before each grazing event, hand-plucked forage samples were collected to determine forage nutritive value. Samples were dried in a forced-air oven at 55°C until constant weight. Dried samples were ground to pass a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific). Ground samples were used to determine the in vitro digestible organic matter (IVDOM) according to the procedure described by Moore and Mott (1974). A subsample was taken from the 2-mm ground sample and ball milled in a Mixer Mill (MM 400, Retsch) at 25 Hz for 9 min. Ball milled samples were analyzed for total N by dry combustion using an elemental analyzer (Vario Micro cube, Elementar) coupled to an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime). Crude protein (CP) was obtained by multiplying the N concentration by 6.25.

Weed proportion and soil cover were estimated following the last grazing cycle in 2018 and 2019. Because of COVID-19 restrictions, measurements of weed proportion and soil cover could not be taken in 2020. Weed proportion was estimated using the dry-weight rank method described by t’Mannetje & Haydock (1963). Briefly, 0.25-m² metallic rings were placed at 20 random points per paddock and the existing plant species were ranked from 1st to 3rd in proportion of total plant biomass. If species had similar proportion at the sampling point, the same rank would be given to both. The proportion of quadrats in which each species appeared as first, second, or third was multiplied by coefficients developed by t’Mannetje & Haydock (1963), i.e., 70.2, 21.1, and 8.7, respectively, in order to convert rank to proportion by weight. As we were not interested in reporting the proportion of each weed species separately, the statistical analysis was performed considering all weeds as a single group. The most common weeds were cutleaf evening primrose (Oenothera laciniata Hill), dandelion (Taraxacum officinale (L.) Weber), red sorrel (Rumex acetosella (L.)), old-field toadflax (Nuttallanthus canadensis (L.) D.A.Sutton), and horse weed (Conyza canadensis (L.)). Soil cover was estimated using the same points used to estimate the weed proportion, by visually grading the proportion of the covered area in each site.

Approximately 10 days after defoliation, cotton bolls were handpicked in 1.8-m length in the two central rows of each plot. Thereafter, cotton was air-dried in greenhouse, and ginned using a saw tooth cotton ginner to determine lint yield.

**Statistical Analysis**

To provide the same number of grazing cycles in each year, the first grazing cycle of 2019 and 2020 was not considered in the statistical analysis. Data were analyzed using PROC GLIMMIX from SAS, ver. 9.4 (SAS Institute, 2013). For pre-and post-grazing HM, IVDOM, and CP, treatments, grazing cycle, and year were considered fixed effects, whereas block was random. For HA rate, treatment, season (early or late), and year were considered fixed effects, and block was considered random. Both, grazing cycles and seasons were analyzed as repeated measures. Soil cover, weed proportion, and cotton lint yield were analyzed with treatment and year as fixed effects and block as a random effect. The LSMEANS were considered statistically different at \( P < 0.05 \) according to the PDIF procedure.

**Results**

**Pre- and Post-grazing Herbage Mass**

There was a treatment \( \times \) grazing cycle \( \times \) year interaction for pre-grazing HM (\( P < 0.01, SE = 193 \)). Except for the first grazing cycle in 2018, all other evaluations had significant differences among treatments. UG resulted in greater pre-grazing HM than MG and OG, while MG generally had greater HM than OG (Figure 5). In 2018, pre-grazing HM was greatest in April, with UG resulting in 3970 kg DM ha\(^{-1}\), whereas MG and UG had 3580 and 2060 kg DM ha\(^{-1}\), respectively.

Except for the fourth grazing cycle (Mar./Apr.) of 2018, UG and MG had similar pre-grazing HM. Additionally, there were no differences in pre-grazed HM between UG and MG in February of 2019 and 2020; however, MG had already been grazed one cycle prior, whereas UG did not have enough HM for
grazing. After grazing events had started for all treatments, differences among treatments were maintained throughout the remainder of the season. Pre-grazing HM for 2019 and 2020 were greatest in March, when UG had 4200 and 3090 kg DM ha⁻¹, respectively.

There was a treatment × grazing cycle × year interaction for pre-grazing HM ($P < 0.01$, SE = 90). Except for February 2018, when UG and MG were not different, UG constantly resulted in greater pre-grazing HM than MG, and MG always resulted in greater pre-grazing HM than OG. Such differences are important to assure that grazing management imposed to cover crops had a statistical significance. Therefore, inferences on different grazing management treatments being applied can be made. The target pre-grazing HM for UG, MG and OG were 2500, 1500, and 500 kg DM ha⁻¹, respectively. Average and considering only the pre-grazing HM after the first grazing was performed in the respective treatment, the pre-grazing HM obtained for UG, MG, and OG were 520, 1350, and 2120 kg DM ha⁻¹, respectively.

There was a treatment × year interaction ($P < 0.0001$, SE = 318) for standing residue (Figure 6). In 2018 and 2020, NG90 and NG34 had similar residue before cover crop termination (on average, 5410 and 6850 kg DM ha⁻¹, for 2018 and 2020, respectively); however, in 2019, NG90 had greater residue than NG34 (7540 vs. 6650 kg DM ha⁻¹, respectively). Both non-grazed treatments had greater residue before cover crop termination than any of the grazed treatments. For all three years, UG had greater residue before termination than MG, and MG had greater residue than OG. The non-grazed treatments had greater residue in 2019 and 2020 than in 2018, nonetheless, no significant difference was reported among years for the grazed treatment. On average, standing residue before cover crop termination was 470, 1320, and 2170 kg DM ha⁻¹, for OG, MG, and UG, respectively.

**Herbage Accumulation Rate and Nutritive Value**

The HA rate was affected by treatment × year ($P < 0.05$) and treatment × season ($P < 0.01$). Compared to 2019 and 2020, there was a greater HA rate in 2018 (Figure 7). Herbage accumulation rate for OG, MG, and UG in 2018 were 54, 90, and 114 kg DM ha⁻¹ d⁻¹, respectively. The UG treatments had similar HA rate in 2019 and 2020 (50 and 53 kg DM ha⁻¹ d⁻¹, respectively); however, OG and MG had lower HA rates in 2019 than 2020 (8 vs. 29, and 22 vs. 45 kg DM ha⁻¹ d⁻¹, for OG and MG, respectively).

During the early season, the HA rate was greatest for UG (105 kg DM ha⁻¹ d⁻¹), followed by MG (81 kg DM ha⁻¹ d⁻¹), whereas OG (34 kg DM ha⁻¹ d⁻¹) had the least HA rate (Figure 8). However, there were no differences among treatments in the late season. During the late season, HA rate was much lower for UG (40 kg DM ha⁻¹ d⁻¹) and MG (24 kg DM ha⁻¹ d⁻¹) compared to the early season, whereas it was consistently low for OG (27 kg DM ha⁻¹ d⁻¹).

There was a treatment × grazing cycle × year interaction for CP ($P < 0.001$, SE = 15). Cover crop CP was usually greater in February than in April, and greater for OG than MG and UG (Figure 9). Differences among treatments were more notable in 2019 and 2020, compared to 2018. In February 2018, CP in February was similar among treatments (280 g kg⁻¹, on average); however, in April 2018, OG had greater CP than UG (160 vs 98 g kg⁻¹), whereas MG (123 g kg⁻¹) was intermediate and it did not differ significantly from either OG or UG. From February to March, CP in OG was consistently greater for OG than UG. Overgrazing also resulted in greater CP than MG in the two first evaluations of the year; however, OG and MG CP values were similar from March and through the remaining season. In February 2019, the CP concentration in OG was 329 g kg⁻¹, compared to 209 and 205 g kg⁻¹ for MG and UG, respectively. By April 2019, CP concentration across treatments was 130 g kg⁻¹, on average.

In 2020, OG resulted in greater CP concentration than UG for all grazing cycles (Figure 8). Except for the first (February) and third (March) grazing cycles in 2020, OG resulted in greater CP than MG. The greatest CP for 2018 and 2019 was in the first grazing cycle of the year, but the greatest CP concentration in 2020 occurred for OG (306 g kg⁻¹) during the second grazing cycle. By April 2020, CP concentration for OG was 185 g kg⁻¹, whereas across treatments CP it was 118 g kg⁻¹, for MG and UG, on average.
In vitro digestible organic matter was affected by treatment × grazing cycle × year \( P < 0.05, \text{SE} = 37 \). In vitro digestible organic matter concentration varied across years and it decreased from the first to the last grazing cycle (Figure 9). Overgrazing often resulted in greater IVDOM compared to MG or UG, although there were a few exceptions where no differences were found among treatments. There were cases where MG was intermediate between OG and UG. Such cases were more frequent in 2018 and less frequent in 2019. In 2018, 2019, and 2020, IVDOM for OG ranged from 670 to 810, 590 to 830, and 710 to 800 g kg\(^{-1}\), respectively. Under UG management, IVDOM concentration ranged from 590 to 730, 490 to 690, and 510 to 720 g kg\(^{-1}\), for 2018, 2019, and 2020, respectively.

**Weed Proportion and Soil Cover**

There was a treatment effect for weed proportion \( P < 0.0001, \text{SE} = 1.8 \). The largest difference was mainly due to weeds (100%) being observed only in the control treatment (Table 2). Among treatments that had cover crops, OG had a greater weed proportion (17%) than both non-grazed treatments (6.5%, on average). Moderate grazing and UG had 8% weeds and they were similar to the OG and the non-grazed treatments.

Soil cover was affected by treatment \( P < 0.05, \text{SE} = 4.3 \), NG90 (76%), UG (75%), NG34 (64%), and MG (64%) resulted in greater soil cover than OG (49%). The control had similar soil cover (58%) to OG, however, it was comprised by weeds. Additionally, NG90 and UG had greater soil cover than the control, whereas NG34 and MG did not differ from control.

**Cotton Lint Yield**

There was a year effect for cotton lint yield \( P < 0.05, \text{SE} = 54 \); however, treatments performed similarly. Cotton lint yield was greater in 2019 (520 kg ha\(^{-1}\)) than in 2018 (330 kg ha\(^{-1}\)), with 2020 being similar to the prior years (430 kg ha\(^{-1}\)).

**Discussion**

**Forage Responses**

Post-grazing HM for each grazing treatment, across grazing cycles and the standing residue before cover crop termination confirmed successful protocol use during the trial to reach target stubble biomass for each grazing treatment. There were exceptions at the beginning of the grazing season, when the OG treatment was delayed, as it did not reach the minimum HM. There were variances associated with the post-grazing HM across years; however, variances within years were expected since an indirect measurement was used to control the stubble height while the animals were grazing.

Franzluebbers & Stuedemann (2007) reported that grazing rye rather than allowing it to accumulate as surface residue had a negative impact on summer grain yield, which may have been related to greater soil compaction from grazing livestock. However, 90% of the available forage was grazed. Lower grazing intensities may have minimized soil compaction effects.

In all cases, non-grazed treatments had greater standing biomass than grazed treatments. After the last grazing cycle, OG, MG, and UG had approximately 470, 1320, and 2170 kg DM ha\(^{-1}\) of standing residue (average across years). The greater residue is associated with decrease in soil erosion and runoff, which occurs primarily because the cover crop and its residue intercepts raindrops and dissipate the energy that can cause interrill erosion (Kaspar & Singer, 2011). The extent cover crops can decrease soil erosion depends on cover crop species, planting density and biomass production (Blanco-Canqui et al., 2015). Therefore, non-grazed cover crops could possibly provide better protection from erosion compared to grazed treatments, especially OG.

The increase in stocking rate and forage utilization decreased the importance of aboveground litter in nutrient cycling relative to excreta (Thomas, 1992; Dubeux, Sollenberger, Mathews, Scholberg & Santos, 2007). Therefore, to obtain the total amount of residue left in the plots after cover crop termination, the existing litter that was deposited throughout the experimental period should also be taken into consideration. During the two first years of the study, 2018 and 2019, residue (on dry matter basis) was greater than the post-grazing HM after the last grazing cycle (Chapter 2). The total amount of
existing litter after cover crop termination for OG, MG, and UG were 1910, 4170, and 5290 kg DM ha⁻¹, respectively. In contrast, the existing litter for NG34 and NG90 were 6900 and 8010 kg DM ha⁻¹, respectively, which was much less variable than all grazed treatments. The greater difference occurs in grazed vs non-grazed systems due to efficiency of management practices and forage species, resulting in different grazing losses (Pedreira et al., 2005; Silveira et al., 2013). Therefore, treatments subjected to grazing resulted in the largest differences between standing residue and existing litter after cover crop termination.

Despite similarities between UG and MG occurring in 2018 and first grazing cycles of 2019 and 2020, pre-grazing HM had similar responses to post-grazing HM, with UG often resulting in greater post-grazing HM than MG, and MG resulting in greater HM than OG (Figure 5). Such result is primarily caused due to the management practices applied, which left greater stubble residue for UG, than MG, and greater stubble residue for MG than OG. Therefore, treatments with more post-grazing HM would naturally result in greater pre-grazing HM in the following grazing cycle. However, another factor affecting the pre-grazing HM was the HA rate. There was treatment × year and a treatment × season interaction for HA rate. During the early season, HA rate was greater for UG than MG, and greater for MG than OG (Figure 8). Treatments grazed at severe intensities would result in lesser leaf area and tiller density than treatments grazed at more lenient intensities, which directly affects plant regrowth. Volesky & Anderson (2007) investigated the effect of clipping at 7-, 14-, and 21-cm stubble heights on four cool-season perennial grasses and reported that the 7-cm stubble residue decreased total dry matter production compared to the other stubble heights in all four species, which could be a function of low tiller appearance and leaf replacement rates due to inadequate photosynthate. Differences in HA rate did not occur in the early season because HA of small grains, such as rye and oat, have greatest HA during January and February, and tend to decrease thereafter, when temperature and daylight increase and the forages reach late maturity stages (Dubeux et al., 2016). Additionally, the treatment × year interaction was caused due to greater cumulative rainfall in February and March of 2018, than in 2019 and 2020. The combined effect of rainfall and season where HA rate was greatest (early season), resulted in greater average HA rate in 2018 compared to the other two years.

In vitro digestible organic matter and crude protein concentrations were usually greatest during the first grazing cycles and decreased towards the end of evaluations. The lower CP and IVDOM in UG compared to OG, and many times MG, occurred because plants that were grazed less intensively reached full development earlier that plants that were grazed more intensively. When plants are fully developed, there is a reduction in cell soluble compounds and protein concentration, as the cell wall content increases (Coleman, Moore & Wilson, 2004).

Nutritive value is defined as the predicted animal response based on chemical composition, digestibility, and nature of digested products, as estimated by in vitro or in vivo chemical analyses (Allen et al., 2011). Sollenberger & Vanzant (2011) stated that nutritive value responses, such as CP and IVDOM, to grazing intensity are less predictable than responses such as forage mass and allowance. A literature review performed by Sollenberger et al. (2009) reported that 98% of studies that evaluated the effect of grazing intensity on forage nutritive value reported no effect (32%) or a positive effect (66%) when increasing grazing intensity (Franzluebbers, Agouridis, Vanzant & Owens, 2009). Contrasting UG and OG, without considering the first grazing cycle of each year, in which UG was not grazed, 67% of the times (8 out 12 grazing cycles), OG resulted in greater CP concentration than UG, whereas no difference between treatments were reported during 33% of the grazing cycles (4 out of 12 grazing cycles). Using the same criteria to investigate the effect of grazing intensity on IVDOM, in 92% of the grazing cycles (11 out of 12), OG resulted in greater IVDOM concentration than UG, whereas only in 2% of the grazing cycles (1 out of 12) no difference was reported.

**Weed Proportion and Soil Cover**
Overgrazing cover crops resulted in 2x greater weed coverage than either of the non-grazed treatments, regardless N fertilization rate, which was correlated with the soil cover. Among cover crop treatments, OG had the least soil cover (49%), which was comparable to the fallow plots (58%). Weed suppression is most effective with early-season canopy development and it is also well correlated with cover crop plant density during the season (Brennan & Smith, 2005). Weed suppression after cover crop termination relies on the amount of residual biomass covering the soil surface (Teasdale, 1996). Cover crop residues from 6000 to 8500 kg ha⁻¹ reduced weed density in the northeastern USA (Mohler & Teasdale, 1993). With adequate soil fertility, rye biomass yields can be superior to 9000 kg ha⁻¹ and provide excellent weed control (Mohler & Teasdale, 1993; Reberg-Horton et al., 2011; Smith et al., 2011). Both, MG and UG, had similar weed proportions and soil cover to ungrazed cover crop treatments. Therefore, grazing cover crops at light intensities did not increase weed proportion or negatively affected soil cover in integrated systems. Maintaining soil cover is an efficient method to avoid soil losses via soil erosion and runoff, which can decrease soil productivity by decreasing water infiltration and soil water holding capacity (Langdale et al., 1991; Zhou, Luukkanen, Tokola & Nieminen, 2008; Zuazo & Pleguezuelo, 2009). Cover crops plant material reduce sediment production from agriculture systems by intercepting the kinetic energy of rainfall droplets and by reducing the amount and velocity of runoff (Dabney, Delgado, & Reeves, 2001; Kaspar & Singer, 2011). The extent by which cover crops will decrease soil erosion depends on biomass production and cover crop species, among others (Blanco-Canqui et al., 2015). However, the relationship between soil cover and erosion reduction is exponential, with minimal changes occurring as soil cover proceed towards 100% (Laflen, Foster & Onstad, 1985). Thus, cover crops grazed at light intensities may provide similar reduction in soil erosion than non-grazed cover crops.

**Cotton Lint Yield**

Our results indicated that short-term (3 years), cover crops could be grazed at various intensities without compromising cotton lint yield. Nonetheless, long-term effects on parameters such as soil organic matter and bulk density may alter the results. Using the land during fallow periods should increase farm diversity and resilience, making it more tolerant to market variability and climatic conditions. The effect of cover crops on following crop yield will depend on a variety of factors, including precipitation, cover crop species, time of planting and termination, and time under cover crop management. Blanco-Canqui et al. (2021) summarized the findings of 17 studies reporting subsequent crop yields after the utilization of cover crops. Cover crops increased following crop yield in nine studies, had no effect in six studies, and reduced crop yields in two studies. Furthermore, a global random-effects meta-analysis evaluated cover crop effects on cotton yield and weed suppression, investigating 10 moderating variables in 104 articles, generating 1117 independent studies over 48 yr. The research indicated that the use of cover crops increased cottonseed and lint yield by 6 and 5%, respectively, while decreasing weed biomass by 20% (Toler, Augé, Benelli, Allen, & Ashworth, 2019).

Regardless of grazing treatment, cotton lint yields were significantly less than Florida average yield (USDA-NASS, 2019). Florida average cotton lint yield in 2019 was 1000 kg ha⁻¹, whereas our average yield was 520 kg ha⁻¹. Additionally, in 2018, cotton lint yield was significantly less than in 2019, which was a direct effect of hurricane Michael on 10 Oct 2018, arriving days before the anticipated cotton harvest. Nonetheless, such values are within the range reported in the literature. For example, grazing a rye cover crop resulted in cotton lint yields ranging from 407 to 1154 kg ha⁻¹, were variability was primarily due to weather conditions (Schomberg et al., 2014).

**Summary and Conclusions**

Over a 3-yr study period in north Florida, cotton lint yield was not affected by the addition of rye and oat as cover crops and imposing various management options, including N fertilization rates and grazing intensity. Less intensive grazing of cover crops resulted in greater herbage accumulation than
overgrazing, however, nutritive value responses often followed an inverse pattern and it decreased along grazing cycles. Overgrazing resulted in greater weed proportion and lesser soil cover than non-grazed cover crops, whereas there was no difference between non-grazed cover crops and cover crops grazed at moderate and low intensity. Grazing rye-oat cover crops at moderate intensity (targeting 1500 kg DM ha\(^{-1}\)) appears to be an option to increase land use efficiency during cotton off-season without affecting soil cover.
Table 1. Herbicides used for controlling weeds in cotton stands.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Application Rate† (L ha⁻¹)</th>
<th>Year</th>
<th>Year</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engenia</td>
<td>0.15</td>
<td>31 May, 3 July</td>
<td>29 May, 26 July</td>
<td>12 June, 16 July</td>
</tr>
<tr>
<td>Roundup</td>
<td>0.28</td>
<td>31 May, 3 July</td>
<td>29 May, 26 July</td>
<td>12 June, 16 July</td>
</tr>
</tbody>
</table>

†Commercial formula.
Table 2. Treatment effect on weed proportion and soil cover of rye-oat cover crops grazed at different intensities.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weed (%)</th>
<th>Soil Cover (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Cover Crops</td>
<td>100a†</td>
<td>58bc</td>
</tr>
<tr>
<td>Cover Crops, No Grazing + 34 kg N ha⁻¹</td>
<td>6c</td>
<td>64ab</td>
</tr>
<tr>
<td>Cover Crops, No Grazing + 90 kg N ha⁻¹</td>
<td>7c</td>
<td>76a</td>
</tr>
<tr>
<td>Cover Crops, Overgrazing + 90 kg N ha⁻¹</td>
<td>17b</td>
<td>49c</td>
</tr>
<tr>
<td>Cover Crops, Moderate Grazing + 90 kg N ha⁻¹</td>
<td>8bc</td>
<td>64ab</td>
</tr>
<tr>
<td>Cover Crops, Undergrazing + 90 kg N ha⁻¹</td>
<td>8bc</td>
<td>75a</td>
</tr>
<tr>
<td>Standard Error</td>
<td>1.8</td>
<td>4.3</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

†Means followed by a common letter within column are not significantly different by at the 5% level of significance.
Figure 4. Monthly weather conditions at North Florida Research and Education Center (NFREC) Marianna, FL, during the experimental years.
Figure 5. Treatment $\times$ grazing cycle $\times$ year interaction on pre- ($P < 0.01, \text{SE} = 193$) and post-grazing herbage mass ($P < 0.01, \text{SE} = 90$) of rye-oat cover crops grazed at different intensities. *Indicates significant difference among treatments within year at the 5% level of significance. †Moderate grazing and Undergrazing were not grazed. ‡Undergrazing was not grazed. Bars refer to the standard error.
Figure 6. Treatment x year interaction ($P < 0.0001$, $SE = 318$) on herbage mass before termination of rye-oat cover crops grazed at different intensities. †Means followed by a common letter within year are not significantly different by at the 5% level of significance. Bars refer to the standard error.
Figure 7. Treatment × year interaction ($P < 0.05$; SE = 5.8) on herbage accumulation rate of rye-oat cover crops grazed at different intensities. *Indicates significant difference among treatments within year at the 5% level of significance. Bars refer to the standard error.
Figure 8. Treatment × season interaction ($P < 0.01; SE = 6.4$) on herbage accumulation rate of rye-oat cover crops grazed at different intensities. *Indicates significant difference among treatments within year at the 5% level of significance. Bars refer to the standard error.
Figure 9. Treatment × grazing cycle × year interaction ($P < 0.001$, SE = 15) on crude protein and in vitro digestible organic matter ($P < 0.05$, SE = 37) of rye-oat cover crops grazed at different intensities. *Indicates significant difference among treatments within year at the 5% level of significance. Bars refer to the standard error.
ii) Above and belowground litter decomposition of cover crops grazed at different intensities

Summary: Grazing cover crops may increase land-use efficiency while promoting sustainability. We investigated how grazing intensity affects cover crop litter quantity, quality, decomposition, and the effect on cotton (Gossypium hirsutum L.) N removal. Cover crops were a mixture of rye (Secale cereale L.) and oat (Avena sativa L.), managed as follows: no grazing + 34 kg N ha⁻¹ (NG34), no grazing + 90 kg N ha⁻¹ (NG90), overgrazing (OG), moderate grazing (MG), and undergrazing (UG). Grazed treatments received 90 kg N ha⁻¹. After cover crop termination, above and belowground litter were collected and incubated in situ to estimate decomposition patterns. Samples were removed after 0, 4, 8, 16, 32, 64, and 128 incubation days concomitant with cotton plants used to estimate N removal and synchronism between N release from litter and uptake from cotton. By day 128, NG34 had 87% of its initial aboveground biomass, whereas OG had 58%, driven by greater initial C:N ratio in NG34 than OG (71 vs. 27). Overgrazing had lesser aboveground litter N than NG90 (27 vs. 60 kg N ha⁻¹), and lesser aboveground final N stock than NG90 and UG (16 vs. 47 and 41 kg N ha⁻¹). Compared to aboveground, belowground litter contributed 2-fold to N deposition on average (46 vs. 98 kg N ha⁻¹). Belowground N disappearance was greater for NG90 than NG34 (39 vs. 21 kg N ha⁻¹). Cotton N removal at day 128 was similar across treatments (191 kg N ha⁻¹). Cover crops can be grazed at moderate and light intensities without affecting their performance and N supply for the following crop.

Methods

Experimental Site

The experiment was conducted from June to October of 2018 and 2019 at University of Florida, Institute of Food and Agricultural Sciences, North Florida Research and Education Center, Marianna, FL (30°52’ N, 85°11’ W). The soil at the experiment site was a Red Bay fine sandy loam (Fine-loamy, kaolinitic, thermic Rhodic Kandiudults; USDA Soil Survey Staff, 2020). Weather data during the experiment years are presented in Figure 10. Initial soil samples taken in May 2017 reported pH_water of 6.1, 7.9 g kg⁻¹ of soil organic matter, and Mehlich-1-extractable P = 12.5 g kg⁻¹, K = 36.5 g kg⁻¹, Mg = 57.5 g kg⁻¹, Ca = 358 g kg⁻¹, S = 10 g kg⁻¹, B = 0.16 g kg⁻¹, Zn = 0.9 g kg⁻¹, Mn = 30.5 g kg⁻¹, Fe = 8 g kg⁻¹, and Cu = 0.25 g kg⁻¹.

Sampling Site, Experimental Design, and Grazing Management

‘Florida 401’ rye and ‘RAM’ oat were planted at a rate of 56 kg ha⁻¹ each on 1 Dec. 2017 and 20 Nov. 2018 in plots that measured 7.3 x 15.2 m. Treatments were replicated three times in a randomized complete block design and were as follows: no grazing + 34 kg N ha⁻¹ (NG34), no grazing + 90 kg N ha⁻¹ (NG90), overgrazing (OG), moderate grazing (MG), and undergrazing (UG). All grazed treatments were fertilized with 90 kg N ha⁻¹. 'Florida 401' rye and 'RAM' oat were planted at a rate of 56 kg ha⁻¹ each on 1 Dec. 2017 and 20 Nov. 2018 in plots that measured 7.3 x 15.2 m. Treatments were replicated three times in a randomized complete block design and were as follows: no grazing + 34 kg N ha⁻¹ (NG34), no grazing + 90 kg N ha⁻¹ (NG90), overgrazing (OG), moderate grazing (MG), and undergrazing (UG). All grazed treatments were fertilized with 90 kg N ha⁻¹. The cover crop fertilizer was split-applied at approximately 3-wk after planting and after the first grazing event. Except for the control, all treatments were fertilized with 34 kg N ha⁻¹, 17 kg P ha⁻¹, 74 kg K ha⁻¹, and 3.4 kg B ha⁻¹ in the first fertilization. During the second fertilization, except for the control and NG34, all treatments were fertilized with 56 kg N ha⁻¹.

Plots were grazed every 14 days using Angus heifers (Bos taurus taurus) to maintain the target stubble residue treatments of 500 (OG), 1500 (MG), and 2500 kg DM ha⁻¹ (UG). Livestock average body weight (kg) and standard deviation at the beginning of the grazing season in 2018 and 2019 were 509 ± 30 and 411 ± 39, respectively. The first grazing occurred when the forage mass was 500 kg DM ha⁻¹ greater than the OG target stubble residue (i.e., 1000 kg DM ha⁻¹), which occurred on 15 Feb. 2018 and on 7 Feb. 2019. The double-sampling technique (Haydock and Shaw, 1975) was used to estimate pre- and post-grazing forage mass. The technique consists of regressing aluminum disk settling heights (indirect measurement) and forage mass harvested in the area (0.25 m²; direct measurement). The equations were calibrated every 28-d using 24 paired samples. Before grazing, disk settling heights (30
Disk settling heights were also taken during the grazing event. Once the target height was reached, the animals were removed from the plots.

**Litter Mass and Decomposition**

Cover crops were terminated on 17 Apr. 2018 and 2019, immediately after the last grazing event, by applying 4.7 L ha⁻¹ of glyphosate (Buccaneer Plus®; N-(phosphonomethyl)glycine). After termination, cover crops were mowed using a Woods Bush hog rotary mower. In 2018, the plots were mowed on 7 May; in 2019, they were mowed on 25 Apr. After mowing, the aboveground (shoots) and belowground material (roots) were collected within a week of mowing to prepare for the decomposition trial.

The aboveground plant material was collected by sampling six 0.25-m² quadrats per plot. Samples were then dried for 72 h at 55°C and weighed to estimate total litter dry mass. Approximately 12 g of sample (on a dry matter basis) were placed in Ankom bags (10 x 20 cm, 50µ-porosity; ANKOM Technology) and sealed, according to Dubeux et al. (2006). For each treatment, bags were incubated in situ in the field in the same plots from where they originated. Bags were incubated in duplicate and removed from the field after 0, 4, 8, 16, 32, 64, and 128 d. Additionally, empty bags (three bags per time per block) were placed in the field and removed at the same time points, to serve as correction factors.

The belowground litter (roots) was sampled by using a standard golf hole cutter (Standard Golf Company). Six 0- to 20-cm-depth × 10.8-cm-diam. soil cores (soil + roots) were collected from each plot after cover crop termination and mowing. Three cores in the rows and three cores between rows were collected. Soil cores (soil + roots) were then dried to constant weight at 55°C in a forced-air drying oven and the weight recorded. Soil cores were washed in an 850-µm sieve to remove the soil and foreign material and by repeated decantation from a container onto the sieve. After the adhered soil was removed, the roots were dried using the previously described protocol. The root mass per unit soil mass was recorded. The root mass per hectare was estimated on an organic matter basis, using a 0- to 20-cm-depth soil layer, and bulk density was determined by the undisturbed core method (Grossman & Reinsch, 2002). Subsamples were ground to pass a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific). Ground subsamples were combusted in a muffle furnace at 600°C for 4 h to express the initial root mass on an organic matter (OM) basis.

Since less belowground material was available for testing, approximately 1 g of sample (on a dry matter basis) was placed in Ankom bags (5 x 10 cm, 50-µm porosity; ANKOM Technology) and sealed. For each treatment, bags were incubated (buried) in situ in the field at 10-cm depth in the same plots from where they originated. Bags were removed from the field after 0, 4, 8, 16, 32, 64, and 128 d. Additionally, empty bags (two bags per time per block) were placed in the field and removed at same time points, to use as correction factors. For aboveground and belowground litter, incubations were initiated on 11 June 2018 and 3 June 2019. After removal of the in-situ bags from the field, samples and empty bags were dried at 55°C for 72 h, cleaned with a nylon brush, and weighed. Thereafter, samples were ground to pass through a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific) and analyzed for DM and OM. Subsamples of the 2-mm ground samples were ball milled in a Mixer Mill (MM 400, Retsch) at 25 Hz for 9 min. Ball-milled samples were analyzed for C and N by dry combustion using an elemental analyzer (Vario Micro cube, Elementar). Additionally, only for the aboveground material, samples ground at 2 mm were used to determine acid detergent fiber (ADF) to obtain the material to perform the acid detergent insoluble nitrogen (ADIN). The N concentration in ADF samples was determined with the same protocol described above.

Remaining biomass of each component was calculated using Equation 2-1:

\[
\text{Remaining biomass} (%) = \frac{F \times OM(\%) \text{ at time } x}{I \times OM(\%) \text{ at time } 0} \times 100
\]
Where $F$ is the sample weight after incubation, $I$ is the sample weight at incubation time 0, $time x$ is the day that the bags were removed from the field, and $time 0$ is the incubation day. Remaining N of each component was calculated using Equation 2-2:

$$Remaining \, N \, (\%) = \frac{F \times N(\%) \, at \, time \, x}{I \times N(\%) \, at \, time \, 0} \times 100$$

(2-2)

Remaining biomass, remaining N, C:N ratio and ADIN were analyzed using the PROC GLIMMIX from SAS, with treatment and days of incubation as fixed effects, and years and blocks as random effects. Days of incubation were considered repeated measures. Means were compared using the PDIF procedure adjusted by Tukey’s test at the 5% significance level. When treatment or the interaction treatment $\times$ day of incubation were statistically significant in the ANOVA, nonlinear models were tested to fit the data for each variable and treatments. Nonlinear models were selected for a given response, based on data distribution and type of response. If only days of incubation was significant, the same model was applied for all treatments.

Remaining biomass (OM basis), remaining N, and C:N ratio were explained by the single exponential decay model (Wagner and Wolf, 1999; Dubeux, Sollenberger, Interrante, Vendramini & Stewart, 2006; Silva et al., 2015). The equation describing this process is:

$$X = B_0 \exp^{-kt}$$

(2-3)

where $X$ is the remaining biomass, remaining N, or C:N ratio at day $t$, $B_0$ is the disappearance coefficient, and $k$ is the relative decay rate (g g$^{-1}$ d$^{-1}$). The model used to describe the ADIN was a two-stage model “linear plateau” (McCartor and Rouquette, 1977; Silva et al., 2015). The equation describing this process is:

$$X_t = A + b_1 \times t \quad \text{if} \quad t \leq T$$

and,

$$X_t = A + b_1 \times T \quad \text{if} \quad t > T$$

(2-4)

where $X$ is the concentration of ADIN, $t$ is the day of incubation, $A$ is the initial concentration, $b_1$ is the rate of increase in concentration from the beginning of incubation until plateau is reached; and $T$ is the day in which concentration reaches the plateau.

Litter N was estimated on a dry matter basis by multiplying the N concentration by the dry matter mass and adjusted by the nonlinear model obtained from the remaining N at day 0. The N stock was obtained by fitting the nonlinear model to the litter N at day 128. Nitrogen disappearance was estimated as the difference between the litter N and N stock.

Litter mass, litter initial chemical composition, litter N, N disappearance, and N stock were analyzed using the PROC GLIMMIX from SAS, with treatments as fixed effect, and block and year as random effects. Means were compared using the PDIF procedure adjusted by Tukey’s test at the 5% significance level.

**Cotton Management and Sampling**

Six rows of ‘Delta Pine 1646’ cotton (dicamba resistant) were planted in each plot on 17 May 2018 and 8 May 2019 at 10 seeds per linear meter using a cotton planter (Hayvan no till drill, AG-Meier Industries, LLC, Belton, TX, USA). Weeds were controlled in the paddocks using Engenia® (dicamba: N,N-Bis-(3-aminopropyl)methylamine salt of 3,6- dichloro-o-anisic acid) and Roundup PowerMAX II (Glyphosate, N-(phosphonomethyl)glycine, in the form of its potassium salt) as described in Table 3.

Cotton was fertilized twice each year. On 8 June 2018 and 11 June 2019, the plots were fertilized with 17, 22, and 84 kg ha$^{-1}$ of N, P, and K, respectively. In the second fertilization, only N was applied at a rate of 72 kg N ha$^{-1}$ and it occurred on 25 June 2018 and 31 July 2019.
At the initial day of incubation (day 0) and at each time point that litter bags were collected from the field (4, 8, 16, 32, 64, and 128 days), four representative cotton plants, excluding roots, were manually removed from the plots. Thereafter, cotton plants were dried to a constant weight at 55°C in a forced-air drying oven and the whole dry plant weight was recorded. After drying, samples were chopped using garden scissors and then ground to pass a 1-mm screen using a Brook Crompton Series 2000 Mill (Brook Crompton, Huddersfield, UK). Subsamples of the 1-mm ground samples were ball milled in a Mixer Mill (MM 400, Retsch) at 25 Hz for 9 min. Ball milled samples were analyzed for N by dry combustion using an elemental analyzer (Vario Micro cube, Elementar). The cotton stand was estimated by counting the plants at a 1.8-m length in the two center rows of each plot. Plant population was estimated using the stand and row spacing. Total cotton biomass was estimated using the plant population and the average plant biomass. Nitrogen removal via cotton uptake was estimated by multiplying the aboveground cotton N concentration by its aboveground plant biomass.

Nitrogen removal via cotton uptake was analyzed using the PROC GLIMMIX from SAS, with treatments as fixed effect, and block and year as random effects. Means were compared using the PDIFF procedure adjusted by Tukey’s test at the 5% significance level.

Results

Aboveground Litter Responses

There were significant differences among treatments for initial N concentration, C:N ratio, ADIN, and proportion of ADIN in N (Table 4). Nitrogen concentration was greater for OG (12.4 g kg⁻¹) than NG34 (6.0 g kg⁻¹) and NG90 (7.0 g kg⁻¹). The MG and UG (10.0 g kg⁻¹, average) had greater N concentration than NG34; however, they did not differ from NG90 (7.0 g kg⁻¹). At the beginning of the study, all grazed treatments had lesser C:N ratio than NG34, whereas the UG treatment was intermediate between them and the non-grazed treatments.

Acid detergent insoluble N was greater for OG (4.7 g kg⁻¹) than all other treatments. In addition, MG and UG had similar ADIN (2.8 g kg⁻¹) and they were greater than non-grazed treatments. The proportion of ADIN in N was also greater for OG than the non-grazed treatments. The MG (27%) and UG (27%) treatments had greater ADIN/N than NG34 (14%), nonetheless, they did not differ from NG90 (20%)

Litter mass at the beginning of the study was greater for NG90 (8010 kg DM ha⁻¹) than for any of the other grazed treatments (Table 5). Besides not differing from UG (5290 kg DM ha⁻¹), NG30 (6900 kg DM ha⁻¹) had greater litter mass than MG (4170 kg DM ha⁻¹) and OG (1910 kg DM ha⁻¹). Aboveground litter N was also greater for the NG90 (60 kg N ha⁻¹) than OG (27 kg N ha⁻¹), with all other treatments being intermediate between them.

There was no difference among treatments for N disappearance (10.4 kg N ha⁻¹, on average). Nonetheless, final N stock was greater for NG90 and UG (47 and 41 kg N ha⁻¹, respectively) than OG (16 kg N ha⁻¹).

There was a treatment × day interaction for aboveground remaining biomass (P < 0.0001; SE = 4) and the data was explained by the single negative exponential model (Figure 11). The difference occurred due to the OG and MG treatments (23%, on average) had lesser aboveground biomass than all other treatments (41%, on average) at the end of 128 days of incubation. The average relative decomposition rate (k) for OG and MG were 0.0105 g g⁻¹ d⁻¹, whereas the average relative decomposition rate for NG34, NG90, and UG was 0.0062 g g⁻¹ d⁻¹ (Figure 11).

The aboveground remaining N was affected by treatment × day interaction (P < 0.0001, SE = 7) and it followed the single negative exponential model (Figure 12). The interaction occurred due to a change in rank among NG34 and NG90 and UG, which was clearly noted at the end of the incubation period (day 128). At the end of the incubation period, 87% (k = 0.0012 g g⁻¹ d⁻¹) of the aboveground N remained in NG34, whereas 58% was left in OG (k = 0.0040 g g⁻¹ d⁻¹). Besides the change in ranks, no differences among treatments within day of incubation were found.
Carbon-to-N ratio was affected by treatment × day interaction \((P < 0.0001, \text{SE} = 3.4)\) and fitted the single negative exponential model (Figure 13). At the beginning of incubation (day 0), NG34 and NG90 C:N ratio was greater (71, on average) than all grazed treatments (37, on average). Nonetheless, these differences decreased with time. At day 128, there was no difference among the non-grazed treatments and UG. Average C:N ratio for NG34 and NG90 at day 128 was 40, whereas the C:N ratios for OG, MG, and UG were 18, 20, and 26, respectively. The relative decomposition rate for NG34, NG90, OG, MG, and UG were 0.0051, 0.0046, 0.0033, 0.0060, and 0.0039 g g\(^{-1}\) d\(^{-1}\), respectively.

There was a treatment × day interaction for ADIN \((P = 0.0045, \text{SE} = 0.6)\), and its behavior fit the linear plateau model (Figure 14). The first treatment to reach the plateau was the OG (5.40 g kg\(^{-1}\) at day 8), followed by the MG (6.94 g kg\(^{-1}\) at day 58). The UG was the last treatment to reach the plateau (day 93), and it had the greatest ADIN concentration when the plateau was reached (7.93 g kg\(^{-1}\)). The non-grazed treatments, NG34 and NG90, reached the plateau at day 90 and 62, respectively. They also had the least ADIN concentration at the beginning (1.48 and 1.43 g kg\(^{-1}\), for NG34 and NG90, respectively) and at the end of the incubation period (4.88 and 4.81 g kg\(^{-1}\), for NG34 and NG90, respectively).

**Belowground Litter Responses**

There were no differences among treatments for belowground litter N concentration (12.8 g kg\(^{-1}\), on average), C:N ratio (23.4, on average), root biomass (4320 kg OM ha\(^{-1}\), on average), initial root N (98 kg N ha\(^{-1}\), on average), and final N stock (66 kg N ha\(^{-1}\), on average) (Table 6). Nonetheless, belowground N disappearance was greater for NG90 (39 kg N ha\(^{-1}\)) than NG34 (21 kg N ha\(^{-1}\)) (Table 6). Belowground biomass was affected by treatment × day interaction \((P = 0.0012, \text{SE} = 7.5)\), and it followed the single negative exponential model (Figure 15). At the end of the incubation, the OG treatment had 70% of belowground remaining biomass, whereas the UG had 45% (Figure 15). The other treatments were intermediate between OG and UG (Figure 15). The relative decomposition rate for OG and UG were 0.0030 and 0.0056 g g\(^{-1}\) kg\(^{-1}\) (Figure 15). Besides the significant \(P\) value, there were no differences in the mean separation analysis.

Belowground remaining N was not affected by treatment \((P > 0.05)\), however, it was affected by days of incubation \((P < 0.0001; \text{SE} = 4)\) and the N decay was explained by the single negative exponential model (Figure 16). The \(k\) for belowground remaing N was 0.0031 g g\(^{-1}\) d\(^{-1}\); by the end of the incubation period, or 29% of the N had disappeared.

The C:N ratio for the belowground litter was affected by day of incubation \((P < 0.0001)\), and its curve fit the single negative exponential model (Figure 17). The C:N ratio decreased from 21 at day 0, to 17, at day 128, and it had a \(k = 0.00183\ g g^{-1}\ d^{-1}\).

**Nitrogen Removal via Cotton**

There was a harvest date effect for N removal via cotton \((P < 0.0001, \text{SE} = 11)\), however, there was no difference among treatments. Nitrogen removal during the first 16 days ranged from 1 to 8 kg N ha\(^{-1}\) and did not differ among each other. After day 16, N removal increased to 38, 123, and 191 kg N ha\(^{-1}\), at days 32, 64, and 128, respectively (Figure 18).

**Discussion**

**Aboveground Litter Responses**

Litter loss rates were greater belowground, where the environment was more conducive to mineralization. However, unlike aboveground litter, the belowground measurements often did not vary by management treatment. Grazing management impacted aboveground litter. The greater the grazing intensity, the lesser litter mass occurred (Table 5). The OG treatment had the least litter mass (1910 kg DM ha\(^{-1}\)) when cover crops were terminated, whereas NG90 had greater litter mass (8010 kg DM ha\(^{-1}\)) than any of the grazed treatments (Table 5). Both, MG (4170 kg DM ha\(^{-1}\)) and UG (5290 kg DM ha\(^{-1}\)) had greater litter mass than OG, however, they did not differ between each other. Notably, litter mass after cover crop termination was greater than target stubble residue (500, 1500, and 2500 kg DM ha\(^{-1}\), for OG, MG, and UG, respectively). This was because litter mass also included litter senescence and plant
lodging during grazing events along the season, as a result of animal feeding and trampling effects. The non-grazed treatments were not statistically different between them, however, whereas NG34 had similar litter mass (6900 kg DM ha⁻¹) to UG, NG90 had greater litter mass (8010 kg DM ha⁻¹) than UG (5290 kg DM ha⁻¹). Cover crops residue left after termination are important in driving SOM responses and suppressing weeds. Cover crops residues from 6000 to 8500 kg ha⁻¹ reduced weed density in the northeastern USA (Mohler & Teasdale, 1993). With adequate fertility, rye biomass yields can be superior to 9000 kg ha⁻¹ and provide excellent weed control (Mohler & Teasdale, 1993; Reberg-Horton et al., 2011; Smith et al., 2011).

Litter mass directly affected total litter N, as it is a function of N concentration and litter mass. Litter N after cover crops termination was greater for NG90 (60 kg N ha⁻¹) than OG (27 kg N ha⁻¹), with the other treatment laying between them. However, increasing grazing intensity decreases the proportion of nutrients recycled via aboveground litter and increases the proportion of nutrients recycled via excreta (Thomas, 1992; Dubeux, Sollenberger, Mathews, Scholberg & Santos, 2007). Thus, probably the primary pathway of N recycled in OG was animal excreta.

Biomass and N had greater disappearance for treatments with high N concentration, high ADIN concentration, high proportion of N in ADIN, and low C:N ratio than treatments with low N concentration, low ADIN concentration, low proportion of N in ADIN, and high C:N ratio. Initial N concentration and C:N ratio were previously reported as possible indicators of litter decomposition rate (Weider & Lang, 1982; Jahanzad et al., 2016, Singh et al., 2020). Plants with high initial C:N ratio usually present greater amounts of recalcitrant fractions than plants with a low initial C:N ratio (Weider & Lang, 1982). Residue C:N ratio and reciprocal N concentration were described as the best predictors for estimating N mineralization in cover crops (Quemada & Cabrera, 1995). Initial C:N ratio values for OG, MG, and UG ranged from 27 to 44 and were similar to values reported previously in the literature for cereal rye and oat (Jahanzad et al., 2016; Varela, Barraco, Gili, Taboada, & Rubio, 2017; Sievers & Cook, 2018); however, the values for the non-grazed treatments were greater than the values reported by the same authors. The results were expected since the non-grazed rye-oat mixtures in our study had a much greater residue than the ones in the referenced studies. Carbon-to-N ratio and N concentration in cover crops is also affected by the proportion of leaves and stems (Quemada & Cabrera, 1995), which, may explain in part, why OG had greater ADIN than all other treatments. For example, stems of rye and oat have a C:N ratio of 98.9 and 78.8, respectively, whereas their leaves have a C:N ratio of 28.9 and 12.8 (Quemada & Cabrera, 1995). In the same study, Quemada & Cabrera (1995) reported that N concentration of leaves was greater than in stems, for both rye (15.7 vs. 4.6 g kg⁻¹, respectively) and oat (36.6 vs. 5.7 g kg⁻¹, respectively). Low leaf/stem ratio substantially results in low-quality forage material (Buxton & Mertens 1995). Because we did not investigate the proportion of leaves and stems of the litter mass, we can only suggest that leaf proportion decreased as grazing intensity increased.

Quantity and quality of litter mass are affected by grazing management. Increasing grazing intensity results in litter with greater quality than when grazing is managed at lesser intensities (Dubeux, Sollenberger, Vendramini, Stewart & Interrante, 2006). Grazing the rye-oat cover crops at moderate (MG) and high intensities (OG) resulted in greater N concentration and reduced C:N ratio. Initial concentrations of N and ADIN, high N proportion in ADIN, and low C:N ratio were observed for OG and MG (Table 4). Therefore, remaining biomass at the end of the incubation period of the aboveground material for the OG and MG treatments was less than UG and the non-grazed treatments (NG34 and NG90). The MG and UG were similar in all the parameters in the initial chemical composition (Table 4), nonetheless, whereas MG had lower C:N ratio (41) than NG90 (61), the UG (44) treatment did not differ statistically from NG90. Thus, except for the similarity in C:N ratio of UG and NG90, grazed cover crops often resulted in lesser C:N ratio than non-grazed cover crops. High N concentration and low C:N ratio results in greater decomposition rate and N release in the soil (Singh et al., 2020). Ideally, timing
between cover crops termination and following crop planting should consider N release curves from the cover crops to increase the system efficiency (Sierves & Cook, 2018; Singh et al., 2020).

The C:N ratio of all treatments decreased over time, fitting the negative single exponential curve. At the beginning of the incubation period, the non-grazed treatment had greater C:N ratio than all grazed treatments, nonetheless, these differences decreased over time. By the end of the incubation period, NG90 had the greatest C:N ratio, whereas OG had the least, with other treatments being intermediate between them (Figure 13). Decreasing C:N ratio occurs because soluble C decomposes rapidly, whereas the N bound to the fiber slows N losses (Dubeux, Sollenberger, Interrante, Vendramini & Stewart, 2006). The flattening in the curve by the end of the incubation period, probably occurred because, at day 0, OG had a greater concentration of ADIN (4.7 g kg⁻¹) than all treatments, and greater proportion of ADIN in N (36%) than both non-grazed treatments, indicating that it had a greater portion of its N bound to the fiber than other treatments. The ADIN behavior during the incubation period was explained by the “linear plateau” model (Figure 14). The ADIN is the N bound to the fiber component, which reduces microbial access to this pool (Silva et al., 2015). The OG was the first treatment to reach the plateau, at day 8, with ADIN concentration of 5.4 g kg⁻¹. This result was probably caused due to low initial C:N ratio, a high initial ADIN concentration and high proportion of ADIN in N, causing the OG to reach the plateau more rapidly than all other treatments. The last treatments to reach the plateau for ADIN were NG34 (89 d) and UG (93 d). Despite lower C:N ratio in UG (44) compared to NG34 (74), initial ADIN concentration (2.8 vs. 0.9 g kg⁻¹) and proportion of ADIN in N (27 vs. 14%) were greater UG than in NG34 (Table 4). Because NG34 and UG reached the plateau at similar dates, but UG had greater initial ADIN concentration than NG34, final ADIN for UG was greater than NG34 (7.9 vs. 4.9 g kg⁻¹, respectively). The MG and NG90 reached the plateau for ADIN concentration at day 58 and 62, respectively, and they were intermediate between OG, and UG and NG34. Similar to what happened for UG and NG34, because MG had greater ADIN concentration (2.8 vs 1.4 g kg⁻¹) and proportion of ADIN in N (27 vs 20%), the ADIN concentration when the plateau was reached for ADIN was greater for MG than UG90 (6.9 vs. 4.8 g kg⁻¹, respectively). These results suggest that the day the ADIN concentration plateau is reached depends on the combination of initial C:N ratio, ADIN concentration, and proportion of ADIN in N, whereas the ADIN concentration when the plateau is reached relies on initial ADIN concentration and proportion of ADIN in N. The ADIN includes lignified nitrogen and Maillard products and microbial access to this N pool is limited (Goering et al., 1972; Krishnamoorthy, Muscato, Sniffen & Van Soest, 1982; Silva et al., 2015).

Despite greater litter quality present in the grazed treatments, especially in OG, the initial litter mass for this treatment was lower than all other treatments, whereas the litter N for this treatment was less than NG90 (Table 5). The lack of difference among treatments within day of incubation for remaining N, resulted in all treatments having similar N disappearance. Therefore, because treatments had similar N disappearance, the N stock for the OG (16 kg N ha⁻¹) at the end of the incubation period was less than UG (41 kg N ha⁻¹) and NG90 (47 kg N ha⁻¹), whereas MG and NG34 were intermediate and did not differ statistically from the extremes. Thus, the MG is a better management option when envisioning good litter quality and N stock, since NG34 had the greatest C:N ratio value. In addition, the MG would also increase the land utilization, possibly increasing farmers income via animal product added value.

**Belowground Litter Responses**

Treatments had similar initial belowground N concentration (13 g kg⁻¹, on average), C:N ratio (23, on average), root biomass (4320 kg OM ha⁻¹, on average), root N (98 kg N ha⁻¹, on average), and N stock (66 kg N ha⁻¹, on average) (Table 6). Nonetheless, belowground N disappearance was greater for NG90 (39 kg N ha⁻¹) than NG34 (21 kg N ha⁻¹) (Table 6). Belowground litter of rye-oat mixtures in our study resulted in lower C:N ratio than the value reported by Sierves & Cook (2018) for rye (23 vs. 35, respectively). One of the factors that would have contributed to this difference is the N fertilization
applied in our study and the additional N supplied via animal excreta, since the referenced authors neither supplied N nor had grazing animals in their study. Feces and urine play an important role in N cycling, with most of this nutrient returning to the pasture in either way (Whitehead, 1970; Braz et al., 2002; Dubeux, Sollenberger, Mathews, Scholberg & Santos, 2007).

Despite the significant difference reported in the ANOVA, the mean separation did not show differences among treatments within each day of incubation for belowground biomass disappearance (Figure 15). Additionally, remaining N was not affected by treatments (Figure 16). The lack of differences in remaining biomass and N was likely caused by similar initial C:N ratio across treatments (Table 6). Nonetheless, despite similar root N and remaining N, N disappearance was greater for NG90 than NG34 (39 vs. 21 kg N ha\(^{-1}\), respectively), such effect was likely caused because NG90 had an initial N stock of 112 kg N ha\(^{-1}\), whereas NG34 had 83 kg N ha\(^{-1}\). Because the remaining N curve was similar across treatments, the initial N for NG90 resulted in a greater proportion of N disappearance in NG90 compared to NG34.

Nitrogen Removal via Cotton

At the end of the litter incubation period, on average, 191 kg N ha\(^{-1}\) was retained in cotton plants. Differences among harvest dates were expected, as the plants were planted at similar dates to the incubation day. Nonetheless, differences among treatments did not occur. Nitrogen uptake by cotton plants tends to increase as N fertilization increases (Janat, 2008), however, such differences were not observed in our results. Therefore, if the N supply provided by the cover crops were the only source of N, there would be a negative net balance in the N pool. However, it is important to note that in such system, the cotton plants are not exported, and its biomass remains in the plots after harvesting the lint. Lack of response might be due to the short-term nature of the study. Soil responses often occur at slow rates and can be accelerated in no till systems when cover crops are used (Blanco-Canqui, Mikha, Pressley & Claassen, 2011). Even though, two years of study may impair limitations to observe the indirect impact on cotton N content.

Summary and Conclusions

After, 128, NG34 had 87% of its initial aboveground biomass, whereas OG had 58%, driven by greater initial C:N ratio in NG34 than OG (71 vs. 27). Aboveground cover crop litter N supply (N disappearance) was not different across treatments, however, the final N stock for NG90 was three times greater than OG, which provides a prolonged N supply to the system. Cover crop belowground litter N supply during the 128 days of incubation was superior by 18 kg N ha\(^{-1}\) when fertilizing cover crops with 90 kg N ha\(^{-1}\) than when fertilizing with 34 kg N ha\(^{-1}\). Grazing cover crops at the three different grazing intensities (overgrazing, moderate grazing, and undergrazing) did not decrease N supply via belowground biomass decomposition nor affected N stock.

Regardless of management practice applied, the belowground litter had twice as much N content, three times greater N disappearance, and approximately twice as much final N stock than the aboveground litter. Nonetheless, both, above and belowground litter were important in adding N to the system. On average, cover crops (whole plant) added 42 kg N ha\(^{-1}\) to the system. Cover crops fertilized with 90 kg N ha\(^{-1}\) resulted in greater aboveground litter N than OG, and greater N disappearance than NG34. Meanwhile, NG90 had similar litter N, final N stock, and N disappearance, for both, below and aboveground litter, to MG and UG. Nitrogen removal via cotton was not different among treatments, at the end of the incubation period 191 kg N ha\(^{-1}\) was retained in the aboveground portion of cotton plants, nonetheless, we did not estimate how much N was retained in cotton roots. Therefore, we conclude that cover crops can be grazed at moderate and light intensities without affecting cover crop performance and N supply to the following crop, while increasing land utilization and aggregating value via animal product addition.
Table 3. Herbicides used for controlling weeds in cotton stands.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Application Rate† (L ha⁻¹)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engenia</td>
<td>0.15</td>
<td>2018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31 May and 3 July</td>
</tr>
<tr>
<td>Roundup (Power Max II)</td>
<td>0.28</td>
<td>2019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31 May and 3 July</td>
</tr>
</tbody>
</table>

†Commercial formula.
Table 4. Initial chemical composition of aboveground rye-oat litter grazed at different intensities; average of 2 yr and three reps.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N Concentration</th>
<th>C:N (ratio)</th>
<th>ADIN (g kg⁻¹)</th>
<th>ADIN/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Grazing + 34N</td>
<td>6.0c†</td>
<td>71a</td>
<td>0.9c</td>
<td>14c</td>
</tr>
<tr>
<td>No Grazing + 90N</td>
<td>7.0bc</td>
<td>61ab</td>
<td>1.4c</td>
<td>20bc</td>
</tr>
<tr>
<td>Over Grazing</td>
<td>12.4a</td>
<td>27c</td>
<td>4.7a</td>
<td>36a</td>
</tr>
<tr>
<td>Moderate Grazing</td>
<td>10.3ab</td>
<td>41c</td>
<td>2.8b</td>
<td>27ab</td>
</tr>
<tr>
<td>Under Grazing</td>
<td>9.7abc</td>
<td>44bc</td>
<td>2.8b</td>
<td>27ab</td>
</tr>
<tr>
<td>SE</td>
<td>1.0</td>
<td>6</td>
<td>0.8</td>
<td>7</td>
</tr>
</tbody>
</table>

*P*-value: 0.0008 <0.0001 <0.0001 <0.0001

†Means followed by a common letter within column are not significantly different by the PDIF procedure adjusted by Tukey at the 5% level of significance.
Table 5. Litter mass, litter N, N disappearance, and final N stock of rye-oat aboveground litter grazed at different intensities, average of 2 yr.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Litter Mass kg ha⁻¹</th>
<th>Litter N</th>
<th>N Disappearance‡</th>
<th>Final N Stock‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Grazing + 34N</td>
<td>6900ab†</td>
<td>43ab</td>
<td>6a</td>
<td>37ab</td>
</tr>
<tr>
<td>No Grazing + 90N</td>
<td>8010a</td>
<td>60a</td>
<td>12a</td>
<td>47a</td>
</tr>
<tr>
<td>Over Grazing</td>
<td>1910d</td>
<td>27b</td>
<td>11a</td>
<td>16b</td>
</tr>
<tr>
<td>Moderate Grazing</td>
<td>4170c</td>
<td>45ab</td>
<td>10a</td>
<td>35ab</td>
</tr>
<tr>
<td>Under Grazing</td>
<td>5290bc</td>
<td>55ab</td>
<td>13a</td>
<td>41a</td>
</tr>
<tr>
<td>SE</td>
<td>469</td>
<td>10</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td><em>P</em>-value</td>
<td>&lt;0.0001</td>
<td>0.0380</td>
<td>0.2432</td>
<td>0.0035</td>
</tr>
</tbody>
</table>

†Means followed by a common letter within column are not significantly different by the PDIF procedure adjusted by Tukey at the 5% level of significance. ‡After 128 d of incubation.
Table 6. Nitrogen concentration, C:N ratio, root biomass, root N, N stock, and N disappearance of rye-oat roots from pastures grazed at different grazing intensities, average of 2 yr and three blocks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N Conc. g kg⁻¹</th>
<th>C:N Ratio</th>
<th>Root Biomass kg OM ha⁻¹</th>
<th>Root N kg N ha⁻¹</th>
<th>N Stock‡ kg N ha⁻¹</th>
<th>N Disap.‡ kg N ha⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Grazing + 34N</td>
<td>12</td>
<td>25</td>
<td>3580</td>
<td>83</td>
<td>62</td>
<td>21b†</td>
</tr>
<tr>
<td>No Grazing + 90N</td>
<td>12</td>
<td>24</td>
<td>5380</td>
<td>112</td>
<td>73</td>
<td>39a</td>
</tr>
<tr>
<td>Over Grazing</td>
<td>13</td>
<td>22</td>
<td>3790</td>
<td>93</td>
<td>65</td>
<td>27ab</td>
</tr>
<tr>
<td>Moderate Grazing</td>
<td>14</td>
<td>23</td>
<td>4340</td>
<td>98</td>
<td>63</td>
<td>34ab</td>
</tr>
<tr>
<td>Under Grazing</td>
<td>13</td>
<td>23</td>
<td>4500</td>
<td>104</td>
<td>68</td>
<td>36ab</td>
</tr>
<tr>
<td>SE</td>
<td>1.2</td>
<td>2.8</td>
<td>543</td>
<td>16</td>
<td>11</td>
<td>5</td>
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<tr>
<td>P-value</td>
<td>0.6840</td>
<td>0.5316</td>
<td>0.1583</td>
<td>0.4535</td>
<td>0.8405</td>
<td>0.0232</td>
</tr>
</tbody>
</table>

†Means followed by a common letter within column are not significantly different by the PDIF procedure adjusted by Tukey at the 5% level of significance. ‡After 128 d of incubation.
Figure 10. Monthly weather conditions at North Florida Research and Education Center (NFREC) Marianna, FL, during the experimental years.
Figure 11. Aboveground remaining biomass (OM%) of rye-oat cover crops grazed at different intensities, average of 2 yr.
Figure 12. Aboveground remaining nitrogen (DM%) of rye-oat cover crops grazed at different intensities, average of 2 yr and three blocks.
Figure 13. Aboveground C:N ratio of rye-oat cover crops grazed at different intensities, average of 2 yr and three blocks.
Figure 14. Acid detergent insoluble N of rye-oat cover crops grazed at different intensities, average of 2 yr.
Figure 15. Belowground remaining biomass (OM%) of rye-oat cover crops grazed at different intensities; average of 2 yr and three blocks.
Figure 16. Effect of days of incubation on belowground remaining N (%) of rye-oat cover crops grazed at different intensities; average of 2 yr and three blocks.
Figure 17. Effect of days of incubation on belowground C:N ratio of rye-oat cover crops grazed at different intensities; average of 2 yr and three blocks.

C:N Ratio = 0.2124^(-0.0014t)
Figure 18. Effect of harvest date on N removal via aboveground cotton plant when planted after rye-oat cover crops grazed at different intensities, average of 2 yr. †Means followed by a common letter column are not significantly different by the PDIFF procedure adjusted by Tukey at the 5% level of significance.
iii) Sward responses of bahiagrass managed across different grazing intensities in a sod-based rotation system

Summary: Forage quantity and quality, as well as sward persistence, are greatly affected by grazing intensity. Bahiagrass (Paspalum notatum Flüggé) is a rhizomatous perennial warm season grass widely grown in the southern USA. ‘UF Riata’ bahiagrass was a cultivar developed to be less sensitive to daylight variation. There are few studies evaluating above and belowground responses of UF Riata across a variety of grazing intensities. This 3-yr study investigated sward responses of bahiagrass as affected by different grazing intensities. Treatments consisted of three post-grazing herbage mass (HM) imposed by mob stocking every 14 d to reach the targets of 500 (overgrazing, OG), 1500 (moderate grazing, MG), and 2500 (undergrazing, UG) kg DM ha⁻¹. Herbage accumulation (HA) rate was not affected by treatment. Crude protein (CP) was similar among treatments within same harvest date. There was a linear effect of grazing intensity on root-rhizome biomass and soil cover. When moving from UG to OG, root-rhizome biomass and soil cover % decreased linearly (P < 0.05) from 12940 to 9230 kg OM ha⁻¹, and 97 to 93%, respectively. There was a trend on appearance of spontaneous grasses in the OG swards (P = 0.06). At the end of the grazing season, they comprised 34 and 17% of OG and UG swards. Across treatments, proportion of other weeds increased from 2 to 22%, from the first to the last year. When considering both, sward persistence and forage nutritive value, it is more appropriate to use a target post-grazing HM of 1500 kg DM ha⁻¹.

Methods

Experimental Site

The experiment was conducted during three consecutive years, 2018, 2019, and 2020 at University of Florida, Institute of Food and Agricultural Sciences, North Florida Research and Education Center, Marianna, FL (30°52’ N, 85°11’ W). The soil at the experiment site was a Red Bay fine sandy loam (Fine-loamy, kaolinitic, thermic Rhodic Kandiudults) (USDA Soil Survey Staff, 2020). Weather data during the experiment years are presented in Table 7. Initial soil samples taken in May 2017 reported pH_water of 6.1, 7.9 g kg⁻¹ of soil organic matter, and Mehlich-1-extractable P = 12.5 g kg⁻¹, K = 36.5 g kg⁻¹, Mg = 57.5 g kg⁻¹, Ca = 358 g kg⁻¹, S = 10 g kg⁻¹, B = 0.16 g kg⁻¹, Zn = 0.9 g kg⁻¹, Mn = 30.5 g kg⁻¹, Fe = 8 g kg⁻¹, and Cu = 0.25 g kg⁻¹.

Treatments and Experimental Design

‘UF Riata’ bahiagrass was planted on 11 May 2017 at 28 kg seed ha⁻¹ on plots that measured 7.3 x 15.2 m. Treatments applied were three grazing intensities, named here as overgrazing (OG), moderate grazing (MG), and undergrazing (UG). Treatments were laid in a randomized complete block design with three replicates. Grazing intensities were controlled by using cattle (Bos taurus taurus) aiming a target post-grazing HM of 500, 1500, and 2500 kg DM ha⁻¹. The Institutional Animal Care and Use Committee (IACUC) of the University of Florida approved all procedures for the experiment (protocol #202009924).

Experimental Management

The experimental plots were fenced with electric fence and contained individual water trough. Every year, plots were fertilized twice during the experimental period. In the first fertilization, plots were fertilized with 17 kg N ha⁻¹, 22 kg P ha⁻¹, and 83 kg K ha⁻¹, whereas 72 kg N ha⁻¹ was applied during the second fertilization. First and second fertilization in 2018, 2019, and 2020 occurred on 8 June and 25 June, 11 June and 31 July, and on 28 May and 19 June, respectively.

Plots were grazed every 14 days using mob stocking (Allen et al., 2011). Angus heifers (2018 and 2019) and Jersey steers (2020) were used to maintain the target post-grazing HM of 500 (OG), 1500
(MG), and 2500 kg DM ha⁻¹ (UG). Average body weight (kg) and standard deviation for the experimental animals at the beginning of the grazing season in 2018, 2019, and 2020 were 508 ± 30, 445 ± 41, and 518 ± 30, respectively. Grazing occurred when pre-grazing HM was greater than the target post-grazing HM. To avoid periods of excessive heat, grazing occurred from 0430 to 1100 h and from 1630 to 2000 h. During the warmer hours of the day, animals were taken to a resting corral beside the experimental plots, where they had access to shade and water. Between grazing events, animals were on a reserve bahiagrass pasture. On the night prior to grazing events, animals were brought to the resting corral and fasted for 12 h.

The first grazing on 2018, 2019, and 2020 occurred on 21 Apr. 2018, 30 Apr. 2019, and 27 May 2020. Grazing was delayed in 2020 due to COVID restrictions. Instead, plots were mowed at 7.5-cm stubble height on 5 May 2020. In total, there were 13 grazing cycles in 2018, 12 in 2019, and 11 in 2020. To include the same number of evaluations in each year, only 11 grazing cycles from each year were evaluated. Respective dates for each grazing cycle are displayed on Table 8.

The double-sampling technique (Haydock and Shaw, 1975) was used to estimate pre- and post-grazing HM. The technique consists of regressing aluminum disk settling heights (indirect measurement) and forage mass harvested in the area (0.25 m²; direct measurement). The equations were calibrated every 28-d using 24 paired samples. Before grazing, disk settling heights (30 points per paddock) were taken to determine pre-grazing HM using the indirect measurement. Disk settling heights were also taken during the grazing event. Once the target height was reached, the animals were removed from the plots and post-grazing HM was recorded. Two animals would start the grazing in each plot, meanwhile, they could be reallocated to a new plot once target post-grazing HM was reached in their original plot.

Response Variables

Herbage responses

Herbage variables included HM pre- and post-grazing, HA rate, IVDOM, CP, soil cover, spontaneous grass percentage, other weeds percentage, and total weeds percentage. Pre- and post-grazing HM were determined as described above, using the double sampling technique (Haydock and Shaw, 1975). The HA rate was determined during five grazing cycles of each year, by the difference of the pre-grazing HM of the current grazing cycle and the post-grazing HM of the previous cycle, divided by the number of days between grazing cycles. The respective dates of each measurement for HA rate can be found in Table 9.

Before each grazing event, hand-plucked forage samples were collected to determine forage nutritive value. Samples were dried in a forced-air oven at 55°C until constant weight. Dried samples were ground to pass a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific). Ground samples were used to determine the IVDOM according to the procedure described by Moore and Mott (1974). A subsample was taken from the 2-mm ground sample and ball milled in a Mixer Mill (MM 400, Retsch) at 25 Hz for 9 min. Ball milled samples were analyzed for total N by dry combustion using an elemental analyzer (Vario Micro cube, Elementar) coupled to an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime). Crude protein was obtained by multiplying the N concentration by 6.25.

Weed proportion and soil cover

Weed proportion and soil cover were estimated following the last grazing cycle of each year. Weed proportion was estimated using the dry-weight rank method described by t’Mannetje & Haydock (1963). Briefly, 0.25-m² metallic rings were placed at 20 random points per paddock and the existing plant species were ranked from 1st to 3rd in proportion of total plant biomass. If species had similar proportion at the sampling point, the same rank would be given to both. The proportion of quadrats in which each species appeared as first, second, or third was multiplied by coefficients developed by t’Mannetje & Haydock (1963), i.e., 70.2, 21.1, and 8.7, respectively, in order to convert rank to
proportion by weight. Weeds were reported in three distinct ways: spontaneous grasses %, other weeds %, and total weed %, which is the sum of the other two. Spontaneous grasses included mostly crowfoot (*Dactyloctenium aegyptium* (L.) Willd) and crab grass (*Digitaria ciliaris* (Retz.) Koeler), and some common bermudagrass (*Cynodon dactylon* (L.) Pers.). Non-grass weeds were mostly composed by teaweed (*Sida rhombifolia* L.) and pigweed (*Amaranthus spinosus* L.). Soil cover was estimated using the same points used to estimate the weed proportion, by visually grading the proportion of the covered area in each site.

**Root-rhizome responses**

Root-rhizome biomass and N concentration were estimated following the last grazing cycle of each year. Three 0- to 20-cm-depth × 10.8-cm-diam. soil cores were collected from each plot following the last grazing cycle of each year using a golf-hole cutter (Standard Golf Company). Soil cores (root + rhizomes + soil) were dried to constant weight in a forced-air drying oven at 55°C, and the weight was recorded. Then, soil cores were washed in an 850-mm sieve to remove the soil and foreign material and by repeated decantation from a container onto the sieve. After soil removal, roots and rhizomes were dried using the same protocol. The root-rhizome mass per unit soil mass was recorded. The root-rhizome mass per hectare was estimated based on a 0- to 20-cm-depth soil layer and bulk density that was determined using the undisturbed core method (Grossman and Reinsch, 2002) on 10 Oct. 2020. Samples were ground to pass a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific). Ground subsamples were combusted in a muffle furnace at 600°C for 5-h to express the root-rhizome mass on an organic matter (OM) basis. Subsamples of the 2-mm ground samples were ball milled in a Mixer Mill (MM 400, Retsch) at 25 Hz for 9 min. Thereafter, ball milled samples were analyzed for total N by dry combustion using an elemental analyzer (Vario Micro cube, Elementar).

**Statistical Analysis**

Data were analyzed using PROC GLIMMIX from SAS, ver. 9.4 (SAS Institute, 2013). For pre-and post-grazing HM, IVDOM, and CP, treatments, grazing cycle, and year were considered fixed effects, whereas block was random. For HA rate, treatment, period, and year were considered fixed effects, and block was considered random. Both, grazing cycles and periods, were analyzed as repeated measures. Soil cover, total weed %, spontaneous grass %, other weed %, root-rhizome biomass, and root-rhizome N concentration were analyzed with treatment and year as fixed effects and block as a random effect, with year being analyzed as repeated measure. The best covariance matrix for each variable was chosen by using the least corrected Akaike information criterion. Linear contrasts were used to evaluate the effect of treatments on soil cover, total weed %, spontaneous grass %, other weeds %, root-rhizome biomass, and root-rhizome N concentration. Residuals of all variables were checked for normality and homogeneity of variance using the student panel and the PROC UNIVARIATE. The LSMEANS were considered statistically different at $P < 0.05$ according to the PDIFF procedure. For the linear contrasts, trends were declared when $P > 0.05$ and $< 0.10$.

**Results**

**Herbage Responses**

There was a treatment × grazing cycle × year interaction for pre-grazing HM ($P < 0.001$, SE = 217). Except for few exceptions occurring in May-June 2020 and May 2021, when there were no differences among treatments, UG consistently had greater pre-grazing HM than OG, with MG being intermediate between them (Figure 19). Such differences were more prominent from June to Sept. in 2018, from June-July to Oct. in 2019, and from June to Aug. in 2020. The greatest pre-grazing HM in 2018 and in 2020 occurred in June-July, when UG had 5070 and 4040 kg DM ha$^{-1}$, respectively. In 2019, the greatest pre-grazing HM occurred in July, when UG had 6020 kg DM ha$^{-1}$. The least pre-grazing HM for 2018 and 2020 occurred towards the end of the growing season, in Sept. and Oct., respectively, with 500 kg DM ha$^{-1}$, on average. Nonetheless, in 2019, the least pre-grazing HM occurred in May, at the beginning of the growing season, when it was 1010 kg DM ha$^{-1}$.
There was a treatment × grazing cycle × year interaction for post-grazing HM (Figure 20; \( P < 0.001, SE = 100 \)). In 2018 and 2019, differences were consistent across grazing cycles; post-grazing HM was greatest for UG, followed by MG, and then by OG. Such differences were also consistent in 2020, with few exceptions. In May-June of 2020, there were no differences between MG and OG. Conversely, in Sept. and Oct. of 2020, differences between MG and UG did not occur. Across grazing cycles and years, post-grazing HM for UG, MG, and OG was 2200, 1480, and 780 kg DM ha\(^{-1}\) (\( P < 0.001 \)). Besides numerical difference between the target (500, 1500, and 2500 kg DM ha\(^{-1}\)) and real post-grazing HM, differences among treatments throughout grazing cycles and years assured that grazing intensities were successfully applied.

There was no difference among treatments for HA rate, however, it was affected by the grazing cycle × year interaction (Figure 21). Differences among years occurred throughout grazing cycles. In 2018 and 2020, HA rate was greatest in June-July, however in 2020, the value for this period was approximately 60% greater than in 2018 (132 vs. 82 kg DM ha\(^{-1}\) d\(^{-1}\)). In 2019, the greatest HA occurred in Aug.-Sept. (112 kg DM ha\(^{-1}\) d\(^{-1}\)), however, it did not differ significantly from July-Aug. (108 kg DM ha\(^{-1}\) d\(^{-1}\)). Across years, the least HA rate occurred by the end of the growing period, in Sept.-Oct. On average, for 2018 and 2019, HA rate in Sept.-Oct. was 43 kg DM ha\(^{-1}\) d\(^{-1}\), and it was significantly greater than in 2020 for the same period, which was 13 kg DM ha\(^{-1}\) d\(^{-1}\). There was a treatment × grazing cycle × year interaction for crude protein (\( P = 0.037, SE = 7 \)). The interaction occurred because there were changes in ranks and intensities among treatments throughout the season. Nonetheless, treatments did not differ among each other in the same harvest date (Figure 22).

**Weed Proportion and Soil Cover**

There was a treatment linear effect for soil cover (\( P = 0.04, SE = 1.3 \)). Soil cover increased from 93 to 97% when moving from OG to UG, with MG being intermediate between them (Figure 23). Total weed proportion on bahiagrass swards did not vary across treatments or years; on average, total weed contribution at the end of the growing season was 36%. However, when looking separately into the proportion of spontaneous grasses and other weeds, it is possible to observe a trend on the appearance of spontaneous grasses (\( P = 0.06 \)), which was greater in the OG than in the UG, with MG being intermediate among them (Figure 24). Additionally, it was observed that appearance of non-grass weeds was not affected by treatments, nonetheless, they increased over the years (\( P < 0.001 \)). In 2018, the proportion of non-grass weeds in bahiagrass swards was 2%, in 2019 it increased to 8%, and in 2020, non-grass weeds occupied 22% of bahiagrass swards (Figure 25).

**Root-Rhizome Responses**

There was a linear effect (\( P = 0.03, SE = 1790 \)) for grazing intensities on root-rhizome mass of bahiagrass (Figure 26). Overgrazing resulted in the least amount of root-rhizome biomass, with 9230 kg OM ha\(^{-1}\). Conversely, when undergrazed, bahiagrass root-rhizome biomass was 12900 kg OM ha\(^{-1}\). Moderate grazing was intermediate between the other two treatments, with a total of 10990 kg OM ha\(^{-1}\) of root-rhizome biomass.

**Discussion**

**Herbage Responses**

Post-grazing HM was estimated to investigate if differences among grazing intensities were successfully applied. Despite mean fluctuation across grazing cycles and years, differences among treatments in post-grazing HM were observed throughout the experimental period. Target post-grazing HM for OG, MG, and UG was 500, 1500, and 2500 kg DM ha\(^{-1}\), nonetheless, on average, the real value achieved throughout the experimental period, on average, was 780, 1480, and 2200 kg DM ha\(^{-1}\), for OG, MG, and UG, respectively.

Because HA rate was similar across treatments, with few exceptions exclusively occurring during the first or second grazing cycle, pre-grazing HM was also consistent throughout the experimental period, with UG resulting in greater pre-grazing HM than MG, and MG resulting in greater pre-grazing
HM than OG. Stewart Jr. et al. (2007) evaluated herbage responses of continuously stocked bahiagrass under three different grazing intensity managements. Grazing intensity managements in the respective study were a combination of stocking rates (SR) and N fertilization levels and they were defined as low (40 kg N ha\(^{-1}\) yr\(^{-1}\), 1.4 AU ha\(^{-1}\) SR), moderate (120 kg N ha\(^{-1}\) yr\(^{-1}\), 2.8 AU ha\(^{-1}\) SR), and high (360 kg N ha\(^{-1}\) yr\(^{-1}\), 4.2 AU ha\(^{-1}\) SR). Across 4 yr, the authors reported greater HM for low than high intensity (3.42 vs. 2.95 Mg ha\(^{-1}\), respectively), nonetheless HA rate (41 vs. 17 kg ha\(^{-1}\) d\(^{-1}\)), CP (140 vs. 99 g kg\(^{-1}\)), and IVDOM (505 vs. 459 g kg\(^{-1}\)) were greater for high than low. In our grazing intensity approach, using mob grazing to reduce HM to desired stubble residue with a 2-wk. interval, differences among treatments for pre-grazing HM and HA rate did not occur. Nonetheless, there are two main differences between our intensity approach and the one used by Stewart Jr. et al. (2007). Firstly, the variable stocking rate on a continuous stocking system instead of a target stubble residue in a mob grazing to differentiate gazing intensities; secondly, N fertilization levels increased as grazing intensity increased. By keeping similar N fertilization levels and different post-grazing HM, HA was not affected by grazing intensity, therefore as UG was the treatment with greatest post-grazing HM, it also had greatest pre-grazing HM.

Herbage accumulation rate was greater in May-June of 2018 than during the same period in 2019, which can be explained by greater rainfall occurring from May to June in 2018 than in 2019. In June-July, HA rate was greatest for 2020; such effect was driven by the combination of high rainfall and solar radiation during this period. Similar to our results, Stewart Jr. et al. (2007) reported that bahiagrass HA rate tend to be lesser in spring, increasing to maximum during the midsummer, and decreases in late summer to early autumn. However, Inyang et al. (2010) reported differences in HA rate with increasing grazing intensity. The authors investigated herbage responses on Pensacola bahiagrass under continuous stocking when using 4, 8, or 12 heifers ha\(^{-1}\). In the respective study, HA rate had a quadratic effect, with greatest HA (128 kg ha\(^{-1}\) d\(^{-1}\)) rate at 8 heifers ha\(^{-1}\). Herbage accumulation rate in our study were as low as 13 kg DM ha\(^{-1}\) d\(^{-1}\) and as high as 132 kg DM ha\(^{-1}\) d\(^{-1}\), both occurring in 2020, during early fall and early summer, respectively. Despite the long range of variability, such values were similar to those previously reported by Stewart Jr. et al. (2007) and Inyang et al. (2010). Despite the three-way interaction, grazing intensities did not differ in CP within each harvest date, but CP did vary among harvest dates. Crude protein ranged from 74 to 156 g kg\(^{-1}\) and it was within the values reported in the literature (Stewart et al., 2007; Interrante et al., 2009; Vendramini et al., 2013).

**Weed Proportion and Soil Cover**

There was a linear effect of grazing intensity on soil cover percentage, as grazing intensity increased from UG to OG, soil cover decreased from 97 to 93% (Figure 23), however, there was no evidence that such effect was carried over the years (P > 0.05). Previous reports indicate that frequent and intense clipping on bahiagrass have a negative effect on soil cover (Beaty et al., 1970; Interrante et al., 2009). Harvesting UF Riata bahiagrass at 4-cm stubble height using 7-d harvest intervals decreased soil cover to 36%, whereas it was 77% when harvested at 8-cm stubble height and 21-d harvest intervals (Interrante et al., 2009). Maintaining soil cover is an efficient method to avoid soil losses via soil erosion and runoff, which can decrease soil productivity by decreasing water infiltration and soil water holding capacity (Langdale et al., 1991; Zhou, Luukkanen, Tokola, & Nieminen, 2008; Zuazo & Pleguezuelo, 2009).

There was a trend (P = 0.06) in the proportion of spontaneous grasses, which increased from 17 to 34% as grazing intensity increased and soil cover decreased (Figure 24). Vendramini et al. (2013) investigated the effect of two grazing frequencies on four bahiagrass cultivars and reported that soil cover of UF Riata bahiagrass decreased from 92 to 81%, when plots were grazed at 4 and 2-wk. intervals, respectively. Such result is directly related to lesser post-grazing HM and greater percentage of uncovered soil in OG than in the other managements, decreasing the ability to compete with other grasses due to reduction of photosynthetic active tissue, while providing more space for the appearance of spontaneous grasses.
Grazing management is key on maintaining warm-season perennial pastures. Wallau et al. (2016) have shown that weed frequency in limpograss pastures increased as grazing intensity increased, whereas Aguiar et al. (2014) reported that soil cover of ‘Jiggs’ bermudagrass pastures decreased from 95 to 39%, as the stocking rate increased from 3 to 12 AU ha\(^{-1}\). Our results show that when pastures are more intensively grazed, there is a decline in soil cover and bahiagrass may eventually be replaced by spontaneous grasses, especially crowfoot and crab grass. In addition, pastures that are overgrazed may present high percentages of uncovered soil, increasing water runoff, sediment loss, and soil temperatures, possibly culminating in land degradation (Kairis, Karavitis, Salvati, Kounalaki & Kosmas, 2015).

Despite no difference among treatments for the percentage of other weeds in the swards, they increased over the years. Therefore, weed control should be considered regardless of grazing intensity. Meanwhile, bahiagrass is also utilized on sod-based rotation systems, where the perennial pasture is typically terminated after a 2-yr period (Katsvairo et al., 2007), when, according to our results, it could have about 8% of non-grass weeds.

**Root-Rhizome Responses**

Ten yrs. after establishment and without receiving any N fertilization during the last 3 yrs., when samplings took place, Santos et al. (2019a) reported that UF Riata in a similar environment was amongst bahiagrass cultivars with the least root-rhizome biomass. Even though, UF Riata root-rhizome biomass was approximately 14000 kg OM ha\(^{-1}\), when plots were harvested five times a year at 5-cm stubble height. For comparison, root-rhizome biomass of ‘Argentine’ bahiagrass in the same study was 25000 kg OM ha\(^{-1}\). Interrante et al. (2009) also indicated that UF Riata typically had lesser root-rhizome biomass than other bahiagrass cultivars, as Argentine and Pensacola. Vendramini et al. (2013) suggests that because UF Riata was improved for superior HA, it could come with the cost of decreasing reserve structures. In our study, UF Riata showed a negative response on root-rhizome biomass as grazing intensities increased from targeting 2500 to 500 kg DM ha\(^{-1}\) post-grazing HM. Overgrazed bahiagrass had about 73% of root-rhizome biomass present in the undergrazed bahiagrass. Root-rhizome biomass for UG, MG, and OG were 12900, 10990, and 9230 kg OM ha\(^{-1}\), respectively. Vendramini et al. (2013) indicated that root-rhizome mass of UF Riata bahiagrass are sensitive to grazing frequency, decreasing from 2.8 to 2.0 Mg ha\(^{-1}\) when grazed at 4- and 2-wk. intervals, respectively. Nonetheless, besides reporting that UF Riata had lesser root-rhizome biomass than other bahiagrass cultivars, Interrante et al. (2009) did not find differences in root-rhizome biomass of UF Riata when using a 4- or 8-cm stubble height at 7- or 21-d harvest frequency.

**Summary and Conclusions**

Changing target residual post-grazing HM from 2500 to 500 kg DM ha\(^{-1}\) had no effect on bahiagrass herbage accumulation rate, resulting in a linear increase on herbage mass as grazing intensity decreased. However, as grazing intensity increased, root-rhizome biomass declined and the proportion of spontaneous grasses in the sward increased. Weed frequency of non-grass weeds were not affected by grazing intensity, nonetheless, they increased over the years. Crude protein varied with harvest dates, however, within the same harvest date, treatments were not different. This was primarily caused due to a target herbage mass was maintained; therefore, the upper part of the leaves would result in similar crude protein across treatments. When considering both, sward persistence and forage nutritive value, we recommend a target post grazing HM for UF Riata bahiagrass of 1500 kg DM ha\(^{-1}\), when pastures are grazed in a 2-wk interval. When considering bahiagrass as a short-term rotation crop, termination after two years would be best to avoid excess of spontaneous weeds. Additionally, it is possible that a greater amount of root-rhizome biomass would result in greater soil organic C pools.
Table 7. Monthly weather conditions at North Florida Research and Education Center (NFREC) Marianna, FL, during the experimental years.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2018</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Min. temperature, °C</td>
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<td>18</td>
<td>21</td>
<td>20</td>
<td>20</td>
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<td>Max. temperature, °C</td>
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<td>35</td>
<td>35</td>
<td>34</td>
<td>35</td>
<td>33</td>
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<tr>
<td>Rainfall, mm</td>
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<td>144</td>
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<td>Solar Radiation, W m⁻²</td>
<td>211</td>
<td>222</td>
<td>208</td>
<td>182</td>
<td>182</td>
<td>157</td>
</tr>
<tr>
<td><strong>2019</strong></td>
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<td></td>
</tr>
<tr>
<td>Min. temperature, °C</td>
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<td>20</td>
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<td>20</td>
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<td>Max. temperature, °C</td>
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<td>38</td>
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<td>Rainfall, mm</td>
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<td>Solar Radiation, W m⁻²</td>
<td>241</td>
<td>219</td>
<td>218</td>
<td>205</td>
<td>212</td>
<td>142</td>
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<td><strong>2020</strong></td>
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<tr>
<td>Min. temperature, °C</td>
<td>11</td>
<td>17</td>
<td>21</td>
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<td>Max. temperature, °C</td>
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<td>35</td>
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Table 8. Grazing cycles and their respective dates in each year.

<table>
<thead>
<tr>
<th>Grazing Cycle</th>
<th>Period</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2018</td>
</tr>
<tr>
<td>-</td>
<td>24 Apr.§</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>7 May§</td>
<td>30 Apr.§</td>
</tr>
<tr>
<td>1</td>
<td>May</td>
<td>21 May</td>
</tr>
<tr>
<td>2</td>
<td>May-June</td>
<td>4 June</td>
</tr>
<tr>
<td>3</td>
<td>June</td>
<td>18 June</td>
</tr>
<tr>
<td>4</td>
<td>June-July</td>
<td>3 July</td>
</tr>
<tr>
<td>5</td>
<td>July</td>
<td>16 July</td>
</tr>
<tr>
<td>6</td>
<td>July-Aug.</td>
<td>31 July</td>
</tr>
<tr>
<td>8</td>
<td>Aug.-Sept.</td>
<td>28 Aug.</td>
</tr>
<tr>
<td>9</td>
<td>Sept.†</td>
<td>11 Sept.</td>
</tr>
<tr>
<td>10</td>
<td>Sept.‡</td>
<td>25 Sept.</td>
</tr>
</tbody>
</table>

†Early to mid Sept. ‡Mid to late Sept. §Data not included in statistical analysis. ¥Plots were mowed at 7.5-cm stubble height.
Table 9. Periods and respective dates used for herbage accumulation rate estimation.

<table>
<thead>
<tr>
<th>Period</th>
<th>2018</th>
<th>2019</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>May-June</td>
<td>4 June to 18 June</td>
<td>13 May to 27 May</td>
<td>27 May to 10 June</td>
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<tr>
<td>June-July</td>
<td>3 July to 16 July</td>
<td>6 June to 24 June</td>
<td>23 Jun to 7 July</td>
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<tr>
<td>July-Aug.</td>
<td>31 July to 14 Aug.</td>
<td>8 July to 22 July</td>
<td>21 July to 4 Aug.</td>
</tr>
</tbody>
</table>
Figure 19. Treatment × grazing cycle × year interaction on pre-grazing herbage mass ($P < 0.001$, SE = 217) of bahiagrass grazed at different intensities. ns = non-significant. Bars refer to the standard error. †Early to mid Sept. ‡Mid to late Sept.
Figure 20. Treatment × grazing cycle × year interaction on post-grazing herbage mass \((P < 0.001, \text{SE} = 100)\) of bahiagrass grazed at different intensities. Bars refer to the standard error. †Early to mid Sept. ‡Mid to late Sept.
Figure 21. Grazing cycle × year interaction on herbage accumulation rate of bahiagrass. †Means followed by a different letter within period are statistically different at the 0.05 probability level. Bars refer to the SE.
Figure 22. Treatment × grazing cycle × year interaction on herbage mass crude protein ($P = 0.037$, SE = 7) of bahiagrass grazed at different intensities. Bars refer to the standard error. †Early to mid Sept. ‡Mid to late Sept.
Figure 23. Linear effect ($P = 0.04$, SE = 1.3) of grazing intensity on soil cover of bahiagrass swards. Bars refer to the SE.
Figure 24. Linear trend ($P = 0.06$, SE = 6.2) of grazing intensity on appearance of spontaneous grasses in bahiagrass swards. Bars refer to the SE.
Figure 25. Year effect ($P < 0.001$) on proportion of non-grass weeds in bahiagrass swards. †Means followed by a different letter within period are statistically different at the 0.05 probability level. Bars refer to the SE.
Figure 26. Linear effect ($P = 0.03$, SE = 1790) of grazing intensity on root-rhizome biomass of bahiagrass swards. Bars refer to the SE.
iv) **Short-term soil responses to contrasting grazing intensities in integrated crop-livestock systems**

**Summary:** Integrated crop-livestock systems provide an array of benefits to agricultural systems, including resilience to market fluctuation and optimization of land use. A farm approach to integrate crops and livestock is through the adoption of grazed cover crops. Moreover, the addition of perennial grasses into crop rotations may improve soil organic matter (SOM) and decrease N leaching. However, the effect of grazing intensity in such systems is not fully understood. This 2-yr study investigated short-term effects of cropping system [winter cover crops-summer cotton (*Gossypium hirsutum* L.) and winter cover crops-summer bahiagrass (*Paspalum notatum* Flüggé) rotations], grazing intensity (no grazing, over, moderate, and undergrazing), and N fertilization (34 and 90 kg N ha⁻¹), on physical and chemical properties of the soil-surface (0-15 cm) and deep-soil (0-90 cm) under no-till. Preliminary results indicate that treatments containing bahiagrass improved SOM in 1.5 g kg⁻¹ and had greater soil pH (6.2 vs. 5.9) and surface-soil NH₄-N concentration (8.8 vs. 7.0 mg kg⁻¹) compared to winter grazing on cover crops-cotton systems; however, they had significantly lesser P concentration (15 vs. 22 mg kg⁻¹) and mineral associated organic C stock (9.6 vs. 11.2 Mg ha⁻¹). Soil bulk density was greater in the fallow treatment compared to those with cover crops (1.72 vs. 1.49 g cm⁻³, respectively), despite having no grazing. Treatments had no effect on soil-surface aggregate distribution or in nutrient concentration and stocks from 0-90 cm depth. Further investigation is necessary to better understand the role of cropping system and grazing intensities on soil responses in deep soils.

**Methods**

*Experimental Site*

The experiment was conducted at University of Florida, Institute of Food and Agricultural Sciences, North Florida Research and Education Center, Marianna, FL (30°52’ N, 85°11’ W). The soil at the experiment site is a Red Bay fine sandy loam (Fine-loamy, kaolinitic, thermic Rhodic Kandiudults) (USDA Soil Survey Staff, 2020). Weather data during the experiment years are presented in Figure 27. Initial soil samples taken in May 2017 reported pH water of 6.1, 7.9 g kg⁻¹ of soil organic matter, and Mehlich-1-extractable P = 12.5 g kg⁻¹, K = 36.5 g kg⁻¹, Mg = 57.5 g kg⁻¹, Ca = 358 g kg⁻¹, S = 10 g kg⁻¹, B = 0.16 g kg⁻¹, Zn = 0.9 g kg⁻¹, Mn = 30.5 g kg⁻¹, Fe = 8 g kg⁻¹, and Cu = 0.25 g kg⁻¹.

*Treatments and Experimental Design*

Treatments were a combination of cropping system (sod-based rotation – SBR and integrated crop-livestock system - ICL), grazing management (over, moderate, and undergrazing), and N fertilization, totalizing nine treatments. Treatments were applied in plots that measured 7.3 x 15.2 m and they were replicated three times in a randomized complete block design. The cropping system comprised of two distinct phases, winter and summer, as displayed on Table 10.

*Cover Crop Planting and Management*

On 1 Dec. 2017 and 20 Nov. 2018, ‘Florida 401’ rye (*Secale cereale* L.) and ‘RAM’ oat (*Avena sativa* L.) were planted as cover crops in all treatments, except for No Cover, at a rate of 56 kg ha⁻¹ each. Fertilization during the cool-season were split in two applications, approximately 3-wk after planting and after the first grazing event. Except for the control, all treatments were fertilized with 34 kg N ha⁻¹, 17 kg P ha⁻¹, 74 kg K ha⁻¹, and 3.4 kg B ha⁻¹ in the first fertilization. During the second fertilization, except for No Cover and Cover 34, all treatments were fertilized with 56 kg N ha⁻¹.

Cover crops were terminated on 17 Apr. 2018 and 17 Apr. 2019, right after the last grazing event, by applying 4.7 L ha⁻¹ of glyphosate [Buccaneer Plus®; N-(phosphonomethyl)glycine].
termination, cover crops were mowed using a Woods Bush hog, on 7 May 2018, 25 Apr. 2019, and 4 May 2020.  

**Cotton and Bahiagrass Planting and Management**

‘UF Riata’ bahiagrass was planted on 11 May 2017 at 28 kg seed ha⁻¹ on SBR plots. Six rows of ‘Delta Pine 1646’ cotton (dicamba resistant) were planted in each plot on 17 May 2018, 8 May 2019, and 5 May 2020 at 10 seeds per linear meter using a cotton planter (Hayvan no till drill, AG-Meier Industries, LLC, Belton, TX, USA). Weeds were controlled in the paddocks using Engenia® [dicamba: N,N-Bis-(3-aminopropyl)methylamine salt of 3,6-dichloro-o-anisic acid] and Roundup PowerMAX II [Glyphosate, N-(phosphonomethyl) glycine, in the form of its potassium salt] as described in Table 11.

Cotton was fertilized twice each year. On 8 June 2018, 11 June 2019, and 21 May 2020 the plots were fertilized with 17, 22, and 84 kg ha⁻¹ of N, P, and K, respectively. In the second fertilization, only N was applied at a rate of 72 kg N ha⁻¹ and it occurred on 25 June 2018, 31 July 2019, and 19 June 2020. Cotton was defoliated on 29 Oct. 2018, 5 Nov 2019, and 1 Oct 2020 by using a mix of Finish® 6 Pro [(2-chloroethyl)phosphonic acid and 1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid] at a rate of 1.75 L ha⁻¹, DEF 6 (S-S-S-Tributyl phosphorotrithioate) at a rate of 0.74 L ha⁻¹, and Dropp SC [Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) at a rate of 0.12 L ha⁻¹.

Every year, plots were fertilized twice during the experimental period. In the first fertilization, plots were fertilized with 17 kg N ha⁻¹, 22 kg P ha⁻¹, and 83 kg K ha⁻¹, whereas 72 kg N ha⁻¹ was applied during the second fertilization. First and second fertilization in 2018 and 2019 occurred on 8 June and 25 June, and 11 June and 31 July, respectively.

**Grazing Management**

Plots were grazed every 14 days using mob stocking (Allen et al., 2011). Angus heifers (Bos taurus) were used to maintain the target post-grazing stubble residue of 500 (overgrazing), 1500 (moderate grazing), and 2500 kg DM ha⁻¹ (undergrazing). Grazing occurred when pre-grazing herbage mass was greater than the target post-grazing stubble residue. Between grazing events, animals were on a reserve pasture with similar diet, i.e., rye-oat mixture during the cool-season, and bahiagrass during the warm-season.

The double-sampling technique (Haydock and Shaw, 1975) was used to estimate pre- and post-grazing herbage mass. The technique consists of regressing aluminum disk settling heights (indirect measurement) and forage mass harvested in the area (0.25 m²; direct measurement). The equations were calibrated every 28-d using 24 paired samples. Before grazing, disk settling heights (30 points per paddock) were taken to determine pre-grazing herbage mass using the indirect measurement. Disk settling heights were also taken during the grazing event. Once the target height was reached, the animals were removed from the plots. Two animals would start the grazing in each plot, meanwhile, they could be reallocated to a new plot once target post-grazing herbage mass was reached in their original plot.

**Response Variables**

**Soil pH and fertility**

Soil samples were collected on 11 July 2017 and on 18 July 2019. Ten soil cores were removed at random from each experimental unit at 15-cm depth, using a 2-cm diam. soil probe. The 10 subsamples were composited and air-dried in a greenhouse at ambient temperature. Samples were then passed through a 2-mm screen as a standard procedure and a subsample was shipped to the UF/IFAS Analytical Services Laboratory – Analytical Research Laboratory for pH (EPA Method 150.1), K, P (EPA Method 200.7), OM (loss on ignition), NO₃-N (EPA Method 353.2), and NH₄-N (EPA Method 350.1) analysis. **Mineral associated OM-C and particulate OM-C**

Soil POM-C and MAOM-C were determined using the method described by Cambardella and Elliott (1992) with some modification. Briefly, 10 g of air-dried sample was dispersed by submerging it into 30 mL of 5 g L⁻¹ sodium hexametaphosphate solution and placed on an orbital shaking incubator for
15 h at 180 rpm min⁻¹. After dispersion, the samples were sieved and rinsed several times with deionized water to pass through a 53-mm screen. The slurry that passed through the screen contained the MAOM plus silt and clay. The slurry was captured in a plastic container and dried in a forced-air drying oven at 55°C until all the water was evaporated, and the weight was recorded. The fraction retained on the screen contained residual sand and POM. This was also transported to a plastic container and dried overnight in a forced-air drying oven and its weight was recorded. After drying, both fractions were scraped from the plastic containers using a stainless-steel spatula and transported to 20-mL scintillation vials.

The soil fractions were considered to be free of carbonates in the top layer (first 15 cm), hence C analysis results represented the C in the SOM. Mineral-associated OM and POM (plus residual sand) were ball milled to a fine powder at 1500 rpm for 9 min in a mixer mill (MM 400, Retsch, Haan, Germany). Thereafter, approximately 80 to 90 mg of the ball-milled sample was used to determine total C and d^{13}C by dry combustion using an elemental analyzer (Vario Micro cube, Elementar, Germany) coupled to an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime, Manchester, UK). Mineral associated OM-C was calculated using the 0-15 cm bulk density and the proportion of the fraction relative to the initial sample (10 g). The POM-C stock was determined by the difference between total C stock (described below) and MAOM-C stock.

**Carbon and nitrogen aggregate fraction distribution**

The aggregate size separation was performed on the air-dried samples collected from the 0-15-cm depth following the dry sieving method (Dubeux et al., 2006; Silveira et al., 2014). A 100-g soil subsample (< 2 mm) from each plot was sieved for 5 min in a Ro-Tap TM sieve shaker (W.S. Tyler, Mentor, OH) through a series of four stacked sieves to obtain four aggregate fractions: (i) 2000 to 250 μm, (ii) 250 to 150 mm, (iii) 150 to 53 mm, and (iv) < 53 mm. The 2000- to 250-μm fraction corresponded to small macroaggregates, while the 250- to 150- and 150- to 53- mm fractions corresponded to microaggregates. The fraction < 53 mm consisted of silt- plus clay-sized aggregates. Each aggregate fraction was thereafter analyzed for C and N concentrations and d^{13}C, using the method described, previously. Carbon and N stocks were calculated using the 0-15 cm bulk density and the proportion of each aggregate size fraction. Total C stock was determined by summing up the stocks from all aggregate size fractions.

**Carbon and nitrogen mobilization in the soil profile**

Soil samples were collected on 11 July 2017 and on 18 July 2019. Three soil cores were removed at random from each experimental unit at 90-cm depth, using a 2-cm diam. Subsoil probe (PN150 JMC Environmentalist’s Sub-Soil Probe, JMC Soil Samplers, Newton, IW, USA). Soil cores were divided into four different depth increments: (i) 0 – 15 cm, (ii) 15 – 30 cm, (iii) 30 – 60 cm, and (iv) 60 – 90 cm. One soil core was used to determine the soil bulk density at each respective depth, by drying the samples at 105°C for 24h. The other two cores were air-dried in a greenhouse at ambient temperature and thereafter composited into a single sample. Samples were then passed through a 2-mm screen as a standard procedure and subjected to laboratory analysis.

For C, N, and d^{13}C analyses, a subsample was ball milled to a fine powder at 1500 rpm for 9 min in a mixer mill (MM 400, Retsch, Haan, Germany). Thereafter, approximately 80 to 90 mg of the ball-milled sample was analyzed by dry combustion, as described above. Another subsample was shipped to the UF/IFAS Analytical Services Laboratory – Analytical Research Laboratory for NO3-N (EPA Method 353.2) and NH4-N (EPA Method 350.1) analysis. Carbon, N, NO3-N, and NH4-N stocks were calculated using the bulk density at the respective depths.

**Statistical Analysis**

Data were analyzed using PROC GLIMMIX from SAS 9.4 (SAS Institute, 2013). Treatments were considered a fixed effect, while block was considered random. Because initial conditions were expected to affect soil responses, the data from 2017 (baseline) was modeled as covariate. When soil aggregate
fractions and soil depths were present in the model, they were considered fixed effects and analyzed as repeated measures. Residuals were checked for normality and homogeneity of variance using the student panel and PROC UNIVARIATE. Differences were considered statistically different at \( P < 0.05 \) according to the PDIF adjusted by Tukey.

Five single degree of freedom customized contrasts were made using the statement contrasts to compare treatments as groups. Treatment groups were as follows: Cover = Cover 34, Cover 90, SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Cover = No Cover; Grazing = SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Grazing = No Cover, Cover 34, Cover 90; ICL = ICL-Over, ICL-Mod, ICL-Under; SBR = SBR-Over, SBR-Mod, SBR-Under. Differences were considered statistically different at \( P < 0.05 \).

**Results**

**Surface Soil Fertility**

There were no significant differences among treatments for bulk density, K or OM concentrations, where average soil bulk density was 1.52 g cm\(^{-3}\), and K and OM concentration were 76.3 mg kg\(^{-1}\) and 19.2g kg\(^{-1}\), respectively across treatments. Nonetheless, pH, P, and NH\(_4\)N were affected by management (Table 12). Soil pH for SBR-Over was 6.2, and it was greater than the 5.8 reported for Cover 90, ICL-Mod, and ICL-Under. Phosphorous concentration was greater for ICL-Under (23.3 mg kg\(^{-1}\)) than SBR-Mod (13.8 mg kg\(^{-1}\)), with all other treatments being intermediate among them.

There were significant differences for the bulk density, pH, NH\(_4\)N, P, and OM contrasts (Table 13). Cover resulted in lesser bulk density than No Cover (1.49 vs. 1.72 g cm\(^{-3}\)); however, there were no other differences among bulk density contrasts. Soil pH was greater for SBR (6.2) than ICL (5.9) and No Grazing (5.9). SBR resulted in greater NH\(_4\)N concentrations (8.8 mg kg\(^{-1}\)) than No Grazing (7.5 mg kg\(^{-1}\)) and ICL (7.0 mg kg\(^{-1}\)). No Grazing (21 mg kg\(^{-1}\)) and ICL (22 mg kg\(^{-1}\)) resulted in greater P concentrations than SBR (15 mg kg\(^{-1}\)). Additionally, soil OM concentrations under SBR (20.0 g kg\(^{-1}\)) was greater than for ICL (18.5 g kg\(^{-1}\)).

**Mineral Associated OM-C and Particulate OM-C**

There were no differences among treatments for soil total C stock at the 15-cm depth (\( P = 0.46, \ SE = 2.5 \)). Across systems and grazing intensities, total C stock was 15.4 Mg ha\(^{-1}\) (data not shown). Besides a significant \( P \)-value (\( P = 0.048, \ SE = 0.82 \)) being reported for MAOM-C stock, no differences among treatments occurred upon mean separation (Table 14). On average, MAOM-C was 10.5 Mg ha\(^{-1}\), ranging from 8.9 (SBR-Mod) to 12.1 Mg ha\(^{-1}\) (Cover 90 and ICL-Mod). However, regarding the contrasts (Table 15), it is interesting to note that SBR on average had a lower MAOM-C stock than ICL (9.6 vs. 11.2 Mg ha\(^{-1}\)). Conversely, there was no significant difference among treatments (\( P = 0.553, \ SE = 2.0 \)) or contrasts for POM-C, which averaged 4.8 Mg ha\(^{-1}\) (Tables 14 and 15).

There was a significant difference among treatments for \( d^{13}C \) (\( P = 0.001, \ SE = 0.23 \)). The SBR-Over was more enriched with \( d^{13}C \) (-23.5\%) than all other treatments except for SBR-Mod (-23.7\%) and SBR-Under (-24.0\%). Cover 34, Cover 90, and ICL-Under had the most depleted \( d^{13}C \) (-25.2\%); however, it did not differ from No Cover, ICL-Over, and ICL-Mod (Table 14). Particulate OM-\( d^{13}C \) trended similarly to MAOM-\( d^{13}C \), nonetheless, differences among treatments were more evident (Table 14). SBR-Over had a more enriched POM-\( d^{13}C \) (-22.0\%) than all other treatments, except for SBR-Mod (-23.2\%) and SBR-Under (-23.3\%). Cover 34, Cover 90, ICL-Over, ICL-Mod, and ICL-Under had the most depleted \( d^{13}C \) (-26.4\%, on average); however, they did not differ from No Cover (-25.4\%), which was intermediate between them and SBR-Mod and SBR-Under treatments.

For both, MAOM-\( d^{13}C \) and POM-\( d^{13}C \), the contrasts Grazing vs. No Grazing, SBR vs. ICL, and No Grazing vs. SBR were significant (Table 15). The SBR was more enriched with \( d^{13}C \) than ICL, whereas the significance of Grazing vs. No Grazing was driven more so by SBR than ICL.
Carbon and Nitrogen Aggregate Fraction Distribution

Within each aggregate fraction, there was no difference among treatments for aggregate size distribution \((P > 0.05)\). Across treatments, 51, 24, 20, and 5% of the aggregates were located within 250 – 2000, 150 – 250, 53 – 150, and < 53 mm, respectively. Furthermore, there was no treatment effect for N and C concentrations or stock, as well as \(^{13}C\), within aggregate fractions. Rather, except for C stock, such variables were solely affected by aggregate fraction distribution (Table 16).

Carbon concentration increased from 8.0 to 12.6 g kg\(^{-1}\) as aggregate fraction decreased from 250 – 2000 to < 53 mm. In contrast to N stock, C stock was not affected by aggregate fraction, with each fraction containing 3.8 Mg C ha\(^{-1}\), on average. Similarly, N concentration increased from 0.5 to 1.5 g kg\(^{-1}\) as aggregate fraction decreased from 250 – 2000 to < 53 mm. Moreover, N stocks were greater within fractions from 53 to 250 mm (0.37 Mg ha\(^{-1}\), on average), than < 53 mm (0.25 Mg ha\(^{-1}\)), with 250 – 2000 mm being intermediate among them (0.34 Mg ha\(^{-1}\)).

Discussion

Soil Bulk Density, Fertility, and pH

Grazing cover crops can negatively impact crop yields, which oftentimes is attributed to soil compaction by livestock (Franzluebbers & Stuedemann, 2007, Franzluebbers & Stuedemann, 2008; Schomberg et al., 2014). Schomberg et al. (2014) reported that cotton yields declined when animals grazed rye before cotton was planted, compared to when it was not grazed. Nonetheless, in our study, grazing negatively impact soil bulk density at the surface (0 to 15 cm depth). However, leaving the soil uncovered during the cool season resulted in greater soil bulk density than when cover crops were planted. Conversely, other reports have found no difference in soil bulk density when changing from fallow to cover crops (Blanco-Canqui, Holman, Schlegel, Tatarko, & Shaver, 2013; Blanco-Canqui & Jasa, 2019).

Grazing animals affect nutrient cycling in pastures by altering the proportion of nutrients returned via plant litter and animal excreta. Compared to nutrient recycling via litter, nutrient return via animal excreta has a more heterogenous distribution, because animals tend to concentrate excreta deposition in so called “hot spots”, such as nearby water troughs and shaded areas (Mathews et al., 1999; Dubieux, Sollenberger, Mathews, Scholberg & Santos, 2007; Dubieux et al., 2009). Cattle dung is an important mode of returning available P to grasslands. Dubieux, Santos, & Sollenberger (2004) reported that the dung P pool from animals grazing bahiagrass was greater than the combined plant litter and urine P pools. In our study, grazed treatments resulted in lesser soil P concentrations than non-grazed treatments. Additionally, P concentrations in the SBR was less than ICL and No Grazing. These results might reflect P exportation via animal feces in SBR than in ICL and No Grazing. In this case, P exportation from the systems would be through the use of the mob-stocking technique, since animals were not be continuously grazing the paddocks. Forage consumed by the animals would be later exported locations
outside the study site, and exportation would occur to a greater extent in SBR because these plots were grazed throughout the year.

Dubeux et al. (2009) reported that surface-soil K concentrations were greater with increasing grazing intensity (100 vs 63 mg kg⁻¹, for high and low intensities, respectively); however, neither cropping system or grazing intensity influenced surface-soil K concentrations in this study. Soil pH was greater with SBR-Over than Cover 90, ICL-Mod, and ICL-Under. Additionally, when contrasting SBR vs. No Grazing and SBR vs. ICL, we can observe that SBR resulted in greater soil pH than both. Likewise, NH₄-N contrasts had similar responses to soil pH, with SBR resulting in greater NH₄-N concentration than ICL and No Grazing (Table 13). However, treatments followed a different pattern, with SBR-Under resulting in greater NH₄-N concentration than all ICL treatments, in addition to No Cover, Cover 34, and SBR-Over. Blanco-Canqui & Jasa (2019) evaluated the impact of grass and legume cover crops on soil properties after 12 yr of implementation. The authors reported no difference in soil pH for any of them when compared to the control. On the other hand, Dear, Virgona, Sandral, Swan, & Morris (2009) reported that soil pH at 10-cm depth became more acidic with distance from perennial plants (from 0.2 near the base of perennial plant and up to 1.1 pH units in gaps between the perennials or in annual-only swards. The authors found that incorporating perennial pasture species in swards was effective at reducing nitrate leaching and preventing a decline in surface soil pH. Thus, greater surface soil-NH₄-N in SBR systems compared to ICL might be related to greater soil OM and pH on SBR. Much of this response occurs due to root-rhizome systems may possibly reduce nutrient leaching compared to annual crops, such as cotton. For example, Santos et al. (2019a) indicated that UF Riata bahiagrass roots and rhizome mass may be as much as 15 Mg OM ha⁻¹.

**Mineral Associated OM-C and Particulate OM-C**

The land use change from rhizoma peanut (C₃) to bahiagrass (C₄) in 2017 gradually shifted soil d¹³C towards a less depleted signature, particularly in the SBR treatment. Due to the nature of their photosynthetic pathways, C₃ and C₄ plants discriminate differently against ¹³C, with C₃ plants usually having more depleted d¹³C in plant tissues than C₄ plants (Farquhar et al., 1989; Schweizer et al., 1999). Therefore, surface soil under treatments not containing bahiagrass often had a more depleted d¹³C signature. Since POM represents a more labile fraction of SOM and with greater aggregate size fractions than MAOM, differences in d¹³C were more evident in POM than MAOM. Similar to our study, Santos et al. (2019b) found that soil POM-d¹³C under bahiagrass swards was -22.84‰ compared to -25.84‰ under rhizoma peanut swards.

Cropping system nor grazing intensity impacted POM-C. Particulate OM is a labile fraction of the OM, sensitive to land use change and amendments application (Cambardella & Elliott, 1992; Cambardella, Gajda, Doran, Wienhold, & Kätterer, 2001). Therefore, we would expect to find differences among treatments in the short-term. Nonetheless, the lack of response in POM-C might be correlated with relatively high variability of this metric. In five long-term (13–54 yr) field experiments containing different cropping systems, N fertilizer rates, and organic amendments, POM-C had greater variability than TC in aggregate fractions from 0.063 to 0.600 mm, revealing it to be a less reliable option for predicting changes in SOM (Simonsson, Kirchmann, Magid, & Kätterer, 2014). However, the authors emphasized the importance of investigating POM-C responses in shorter-term studies. Over a 2 yr-period, increasing grazing intensity on Tifton 85 bermudagrass (Cynodon spp.) from 24- to 8-cm stubble residue was linearly associated with a decreasing particulate organic C pool (Silveira, Liu, Sollenberger, Follett, & Vendramini, 2013). The ICL had greater MAOM-C than SBR (Table 15). Overall, MAOM-C pool was greater than POM-C pool, which concurs with finds indicating that grasslands tend to store more MAOM-C than POM-C (Cotrufo, Ranalli, Michelle, Six, & Lugato, 2019). Despite recent land use conversion, especially in No Cover, which does not account with grasses during both cool- and warm-seasons, such changes did not alter MAOM-C pool patterns since this fraction is less susceptible to changes.
Carbon and Nitrogen Aggregate Fraction Distribution

Soil aggregate fraction distribution allows evaluating SOC dynamics among different C pools in response to land management. As previously shown, there was no difference among treatments for C and N concentrations or stocks within aggregate size classes. Evaluating two cropping systems, summer grain-winter cover crop and winter grain-summer cover crop, Franzluebbers & Stuedemann (2008) reported that macroaggregate stability in water was unaffected by cover crop grazing management under conventional tillage or no-till systems. Nonetheless the authors indicated differences between tillage management. In our study, no-tillage was used, thus the differences among systems for SOC in the soil aggregate is supposed to demand more time, given that the aggregate stability in the surface layer in no-tillage is usually greater than under conventional tillage (Hamblin, 1980; Veiga, Reinert, & Reichert, 2009). Therefore, differences in SOC aggregate fraction distribution may present itself over a longer term. Crop species impact aggregation and stabilization of SOC in macroaggregates and microaggregates differently, due to the variation in biomass production and root systems or management that impacts production above- and belowground (Six, Bossuyt, Degryze, & Denef, 2004; Veiga et al., 2009). The activity of soil fungi and microorganisms is one of the factors contributing to soil aggregates formation. Six et al. (2004) reported that following the incorporation of fresh residue in the soil, soil fungi and microorganisms produced mucilage resulting in the formation of macroaggregates around coarse intra-aggregate POM (>250 µm). Furthermore, the authors reported that coarse intra-aggregate POM is further decomposed and fragmented into fine (53 - 250 µm) intra-aggregate POM, forming the microaggregate.

Despite the relatively small proportion of soil associated with the <53-µm fraction (5% vs. 51, 24, and 20% of the aggregates for the 250 – 2000, 150 – 250, 53 – 150 mm, respectively), this fraction had greater C and N concentrations. The larger aggregate fraction offers SOC little physical protection against mineralization. However, greater SOC long-term storage is associated with the fine aggregate size fractions (Silveira, Liu, Sollenberger, Follet, & Vendramini, 2013; Silveira, Adewopo, Franzluebbers, & Buonadio, 2014). In this study, the <53-µm fraction was responsible for 21.3% of the SOC across treatments, and it represents an important mechanism of SOC protection in this soil because C stabilization is greater within microaggregates than macroaggregates (Six, Conant, Paul & Paustian, 2002; Silveira, Liu, Sollenberger, Follet, & Vendramini, 2013; Silveira, Adewopo, Franzluebbers, & Buonadio, 2014). It is interesting to note that the larger aggregate size fractions were more enriched in $^{13}$C (less negative $d^{13}$C) compared to <53-µm fraction. The relatively less negative $d^{13}$C in larger aggregate was probably driven by the addition of C$_4$-derived C recently incorporated into SBR systems.

Carbon and Nitrogen Mobilization in the Soil Profile

The relatively short-term nature of this experiment was likely the main reason for the lack of differences among treatments. Evaluating five different systems in North Florida, including native vegetation, peanut-cotton-cotton, and sod-based rotation under grazing or not and under irrigation or not, Rolando et al. (2021) reported that differences in soil organic C concentrations occurred solely in the top 30-cm depth, and SBR systems, especially when not irrigated, resulted in greater concentrations. In this study, the soil C stock was represented to the greatest extent in the surface (0-15 cm depth) soil, accounting for approximately 37% of total C.

Soil N, NH$_4$-N and NO$_3$-N concentrations were also greatest in the surface soil (0-15 cm depth). Perennial pastures can decrease apparent nitrate leaching compared to using annual forages. In a four-year study, significant increases in soil nitrate were observed deeper in the profile under annual swards, while they typically remained low under swards containing perennial pasture species (Dear, Virgona, Sandral, Swan, & Morris, 2009). In a systematic analysis, Abdalla et al. (2019) indicated that the use of cover crops has the potential to decrease N leaching and increase soil organic C but had no effect on direct N$_2$O emissions. After two years of establishment, there was not a significant difference between
fallow and the use of cover crops on mineral N leaching; however, over a longer sampling period (several years) and pre-study legacy soil N depleted, differences might be found.

Conclusions

After two years of establishment, grazing management and cropping system solely affected soil responses in the top 15 cm. Sod-based rotation systems, with grazed rye-oat during the winter and grazed bahiagrass during the summer, resulted in an increase of 1.5 g kg⁻¹ in soil organic matter, compared to grazed rye-oat during the winter and cotton during the summer, regardless of grazing intensity. Additionally, sod-based rotation resulted in greater pH and soil surface NH₄-N compared to integrated crop-livestock system. Nitrogen leaching is one of the causations of soil acidification. Our results have shown that sod-based rotation systems increase soil organic matter, while maintaining high pH and NH₄-N concentration at the soil surface. Such response is probably driven due to the ability of perennial grass root systems in improving soil organic matter. Low P concentration in sod-based rotation systems potentially occurred due to P exportation via animal excreta. A long-term assessment of the systems is necessary to identify soil responses in deeper layers.
Table 10. Planted crop, N fertilization, and target stubble residues during cool- and warm-season of treatments applied in the study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Winter</th>
<th>N Fertilization</th>
<th>Stubble Residue†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Cover</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cover 34</td>
<td>Rye and oat</td>
<td>34 kg N ha⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>Cover 90</td>
<td>Rye and oat</td>
<td>90 kg N ha⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>SBR-Over</td>
<td>Rye and oat</td>
<td>90 kg N ha⁻¹</td>
<td>500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>SBR-Mod</td>
<td>Rye and oat</td>
<td>90 kg N ha⁻¹</td>
<td>1500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>SBR-Under</td>
<td>Rye and oat</td>
<td>90 kg N ha⁻¹</td>
<td>2500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>ICL-Over</td>
<td>Rye and oat</td>
<td>90 kg N ha⁻¹</td>
<td>500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>ICL-Mod</td>
<td>Rye and oat</td>
<td>90 kg N ha⁻¹</td>
<td>1500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>ICL-Under</td>
<td>Rye and oat</td>
<td>90 kg N ha⁻¹</td>
<td>2500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Cover</td>
<td>Cotton</td>
<td>90 kg N ha⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>Cover 34</td>
<td>Cotton</td>
<td>90 kg N ha⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>Cover 90</td>
<td>Cotton</td>
<td>90 kg N ha⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>SBR-Over</td>
<td>Bahiagrass</td>
<td>90 kg N ha⁻¹</td>
<td>500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>SBR-Mod</td>
<td>Bahiagrass</td>
<td>90 kg N ha⁻¹</td>
<td>1500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>SBR-Under</td>
<td>Bahiagrass</td>
<td>90 kg N ha⁻¹</td>
<td>2500 kg DM ha⁻¹</td>
</tr>
<tr>
<td>ICL-Over</td>
<td>Cotton</td>
<td>90 kg N ha⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>ICL-Mod</td>
<td>Cotton</td>
<td>90 kg N ha⁻¹</td>
<td>-</td>
</tr>
<tr>
<td>ICL-Under</td>
<td>Cotton</td>
<td>90 kg N ha⁻¹</td>
<td>-</td>
</tr>
</tbody>
</table>

†Plots were mob-grazed approximately every 14 d to maintain the target stubble residues. SBR = sod-based rotation; ICL = integrated crop-livestock system.
Table 11. Herbicides used for controlling weeds in cotton stands.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>Application Rate† (L ha⁻¹)</th>
<th>2018</th>
<th>2019</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engenia</td>
<td>0.15</td>
<td>31 May, 3 July</td>
<td>29 May, 26 July</td>
<td>12 June, 16 July</td>
</tr>
<tr>
<td>Roundup</td>
<td>0.28</td>
<td>31 May, 3 July</td>
<td>29 May, 26 July</td>
<td>12 June, 16 July</td>
</tr>
</tbody>
</table>

†Commercial formula.
Table 12. Treatment effect on soil bulk density, fertility, and pH across different cropping systems after two years of establishment at 15-cm soil depth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bulk Density</th>
<th>pH</th>
<th>NH₄-N</th>
<th>P</th>
<th>K</th>
<th>OM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g cm⁻³</td>
<td>units</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>g kg⁻¹</td>
</tr>
<tr>
<td>No Cover</td>
<td>1.72</td>
<td>6.0ab</td>
<td>7.1b</td>
<td>19.5ab†</td>
<td>69.1</td>
<td>19.8</td>
</tr>
<tr>
<td>Cover 34</td>
<td>1.46</td>
<td>5.9ab</td>
<td>6.9b</td>
<td>23.0ab</td>
<td>74.5</td>
<td>19.0</td>
</tr>
<tr>
<td>Cover 90</td>
<td>1.45</td>
<td>5.8b</td>
<td>8.6ab</td>
<td>21.0ab</td>
<td>80.3</td>
<td>18.9</td>
</tr>
<tr>
<td>SBR-Over</td>
<td>1.59</td>
<td>6.2a</td>
<td>7.4b</td>
<td>16.0ab</td>
<td>92.7</td>
<td>20.3</td>
</tr>
<tr>
<td>SBR-Mod</td>
<td>1.47</td>
<td>6.2ab</td>
<td>9.0ab</td>
<td>13.8b</td>
<td>67.6</td>
<td>19.9</td>
</tr>
<tr>
<td>SBR-Under</td>
<td>1.51</td>
<td>6.1ab</td>
<td>10.1a</td>
<td>14.0ab</td>
<td>66.9</td>
<td>19.9</td>
</tr>
<tr>
<td>ICL-Over</td>
<td>1.43</td>
<td>6.0ab</td>
<td>6.9b</td>
<td>22.6ab</td>
<td>91.3</td>
<td>18.0</td>
</tr>
<tr>
<td>ICL-Mod</td>
<td>1.60</td>
<td>5.8b</td>
<td>6.8b</td>
<td>18.7ab</td>
<td>72.3</td>
<td>18.1</td>
</tr>
<tr>
<td>ICL-Under</td>
<td>1.42</td>
<td>5.8b</td>
<td>7.3b</td>
<td>23.3a</td>
<td>72.5</td>
<td>19.3</td>
</tr>
</tbody>
</table>

| Standard Error | 0.09 | 0.07 | 0.6 | 2.4 | 11.4 | 0.07 |
| P-value        | 0.35 | < 0.05 | < 0.01 | < 0.01 | 0.30 | 0.296 |

†Means followed by a common letter within column are not significantly different at the 5% level of significance according to Tukey’s test.
Table 13. Contrast effect on soil bulk density, fertility, and pH across different cropping systems after two years of establishment at 15-cm soil depth.

<table>
<thead>
<tr>
<th>Contrast†</th>
<th>Bulk Density</th>
<th>pH</th>
<th>NH₄-N</th>
<th>P</th>
<th>K</th>
<th>OM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover vs. No Cover</td>
<td>0.031</td>
<td>0.407</td>
<td>0.166</td>
<td>0.840</td>
<td>0.463</td>
<td>0.389</td>
</tr>
<tr>
<td>Grazing vs. No Grazing</td>
<td>0.576</td>
<td>0.105</td>
<td>0.348</td>
<td>0.030</td>
<td>0.746</td>
<td>0.996</td>
</tr>
<tr>
<td>SBR vs. ICL</td>
<td>0.654</td>
<td>0.004</td>
<td><strong>0.001</strong></td>
<td><strong>0.001</strong></td>
<td>0.830</td>
<td><strong>0.017</strong></td>
</tr>
<tr>
<td>No Grazing vs. ICL</td>
<td>0.438</td>
<td>0.205</td>
<td>0.202</td>
<td>0.811</td>
<td>0.562</td>
<td>0.198</td>
</tr>
<tr>
<td>No Grazing vs. SBR</td>
<td>0.833</td>
<td>0.010</td>
<td><strong>0.007</strong></td>
<td><strong>0.001</strong></td>
<td>0.933</td>
<td>0.194</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group Mean</th>
<th>g cm⁻³</th>
<th>units</th>
<th>mg kg⁻¹</th>
<th>mg kg⁻¹</th>
<th>mg kg⁻¹</th>
<th>g kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>1.49</td>
<td>6.0</td>
<td>7.9</td>
<td>19</td>
<td>77</td>
<td>19.2</td>
</tr>
<tr>
<td>No Cover</td>
<td>1.72</td>
<td>6.0</td>
<td>7.1</td>
<td>19</td>
<td>69</td>
<td>19.8</td>
</tr>
<tr>
<td>Grazing</td>
<td>1.50</td>
<td>6.0</td>
<td>7.9</td>
<td>18</td>
<td>77</td>
<td>19.2</td>
</tr>
<tr>
<td>No Grazing</td>
<td>1.54</td>
<td>5.9</td>
<td>7.5</td>
<td>21</td>
<td>75</td>
<td>19.2</td>
</tr>
<tr>
<td>SBR</td>
<td>1.52</td>
<td>6.2</td>
<td>8.8</td>
<td>15</td>
<td>76</td>
<td>20.0</td>
</tr>
<tr>
<td>ICL</td>
<td>1.48</td>
<td>5.9</td>
<td>7.0</td>
<td>22</td>
<td>79</td>
<td>18.5</td>
</tr>
</tbody>
</table>

†Cover = Cover 34, Cover 90, SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Cover = No Cover; Grazing = SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Grazing = No Cover, Cover 34, Cover 90; ICL = ICL-Over, ICL-Mod, ICL-Under; SBR = SBR-Over, SBR-Mod, SBR-Under.
Table 14. Treatment effect on mineral associated- (MAOM) and particulate soil organic matter (POM) $d^{13}$C and C stock of different cropping systems after two years of establishment at 15-cm soil depth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$d^{13}$C</th>
<th>C Stock</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>‰</td>
<td>MAOM</td>
<td>POM</td>
</tr>
<tr>
<td>No Cover</td>
<td>-25.1cd†</td>
<td>-25.4bc</td>
<td>10.8</td>
</tr>
<tr>
<td>Cover 34</td>
<td>-25.2d</td>
<td>-26.4c</td>
<td>9.3</td>
</tr>
<tr>
<td>Cover 90</td>
<td>-25.2d</td>
<td>-26.0c</td>
<td>12.1</td>
</tr>
<tr>
<td>SBR-Over</td>
<td>-23.5a</td>
<td>-22.0a</td>
<td>10.4</td>
</tr>
<tr>
<td>SBR-Mod</td>
<td>-23.7ab</td>
<td>-23.2ab</td>
<td>8.9</td>
</tr>
<tr>
<td>SBR-Under</td>
<td>-24.0abc</td>
<td>-23.3ab</td>
<td>9.5</td>
</tr>
<tr>
<td>ICL-Over</td>
<td>-25.0bcd</td>
<td>-27.0c</td>
<td>10.5</td>
</tr>
<tr>
<td>ICL-Mod</td>
<td>-24.9bcd</td>
<td>-26.4c</td>
<td>12.1</td>
</tr>
<tr>
<td>ICL-Under</td>
<td>-25.2d</td>
<td>-26.5c</td>
<td>11.0</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.23</td>
<td>0.46</td>
<td>0.82</td>
</tr>
<tr>
<td>$P$-value</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.048</td>
</tr>
</tbody>
</table>

†Means followed by a common letter within column are not significantly different at the 5% level of significance according to Tukey’s test.
Table 15. Contrast effect on mineral associated- (MAOM) and particulate soil organic matter (POM) d\textsuperscript{13}C and C stock of different cropping systems after two years of establishment at 15-cm soil depth.

<table>
<thead>
<tr>
<th>Contrast†</th>
<th>d\textsuperscript{13}C</th>
<th>C Stock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAOM</td>
<td>POM</td>
</tr>
<tr>
<td>Cover vs. No Cover</td>
<td>0.063</td>
<td>0.495</td>
</tr>
<tr>
<td>Grazing vs. No Grazing</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>SBR vs. ICL</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No Grazing vs. ICL</td>
<td>0.503</td>
<td>0.078</td>
</tr>
<tr>
<td>No Grazing vs. SBR</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Group Mean

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>Mg ha\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td>-24.6</td>
<td>10.5</td>
</tr>
<tr>
<td>No Cover</td>
<td>-25.1</td>
<td>10.8</td>
</tr>
<tr>
<td>Grazing</td>
<td>-24.4</td>
<td>10.4</td>
</tr>
<tr>
<td>No Grazing</td>
<td>-25.2</td>
<td>10.7</td>
</tr>
<tr>
<td>SBR</td>
<td>-23.7</td>
<td>9.6</td>
</tr>
<tr>
<td>ICL</td>
<td>-25.0</td>
<td>11.2</td>
</tr>
</tbody>
</table>

†Cover = Cover 34, Cover 90, SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Cover = No Cover; Grazing = SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Grazing = No Cover, Cover 34, Cover 90; ICL = ICL-Over, ICL-Mod, ICL-Under; SBR = SBR-Over, SBR-Mod, SBR-Under.
Table 16. N and C concentration and stocks, and d^{13}C across different soil aggregate fractions at 15-cm soil depth.

<table>
<thead>
<tr>
<th>Aggregate fraction</th>
<th>N</th>
<th>C</th>
<th>d^{13}C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>Stock</td>
<td>Concentration</td>
</tr>
<tr>
<td>250 – 2000 mm</td>
<td>0.5c†</td>
<td>0.34ab</td>
<td>8.0c</td>
</tr>
<tr>
<td>150 - 250 mm</td>
<td>0.7b</td>
<td>0.37a</td>
<td>8.9bc</td>
</tr>
<tr>
<td>53 – 150 mm</td>
<td>0.8b</td>
<td>0.36a</td>
<td>9.7ab</td>
</tr>
<tr>
<td>&lt; 53 mm</td>
<td>1.5a</td>
<td>0.25b</td>
<td>12.6a</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.07</td>
<td>0.03</td>
<td>0.56</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

†Means followed by a common letter within column are not significantly different at the 5% level of significance according to Tukey’s test.
Table 17. Soil depth effect on nitrogen and carbon responses of different cropping systems after two years of establishment.

<table>
<thead>
<tr>
<th>Soil Depth</th>
<th>NH₄-N</th>
<th>NO₃-N</th>
<th>Nitrogen</th>
<th>Carbon</th>
<th>C:N</th>
<th>d¹³C</th>
</tr>
</thead>
<tbody>
<tr>
<td>cm</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>g kg⁻¹</td>
<td>Mg ha⁻¹</td>
<td>g kg⁻¹</td>
<td>Mg ha⁻¹</td>
</tr>
<tr>
<td>0 – 15</td>
<td>3.7a†</td>
<td>10.6a</td>
<td>0.37a</td>
<td>0.9a</td>
<td>4.1a</td>
<td>11a</td>
</tr>
<tr>
<td>15 – 30</td>
<td>2.5b</td>
<td>5.3b</td>
<td>0.22b</td>
<td>0.5b</td>
<td>2.8b</td>
<td>7b</td>
</tr>
<tr>
<td>30 – 60</td>
<td>2.0b</td>
<td>5.8b</td>
<td>0.18b</td>
<td>0.7a</td>
<td>2.2bc</td>
<td>7b</td>
</tr>
<tr>
<td>60 – 90</td>
<td>2.2b</td>
<td>4.8b</td>
<td>0.16b</td>
<td>0.6ab</td>
<td>1.9c</td>
<td>5c</td>
</tr>
<tr>
<td>SE</td>
<td>0.4</td>
<td>0.7</td>
<td>0.03</td>
<td>0.07</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

†Means followed by a common letter within column are not significantly different at the 5% level of significance according to Tukey’s test.
Figure 27. Monthly weather conditions at North Florida Research and Education Center (NFREC) Marianna, FL, during the experimental years.
v) Nitrate leaching in contrasting cropping systems

Leachate water collections from drain lysimeters (Drain Gauges) at 5 ft (1.5 m) depth, allowed for the calculation of potential water and inorganic N (almost exclusively NO₃-N) leaching losses from crop and grazing systems. Additionally, rainfall/irrigation and evapotranspiration (ET₀) tracking (from Florida Automated Weather Network; FAWN) provided reference values that aided in identifying times of heightened N leaching loss from agricultural systems. Drain gauges were installed in winter, 2018. As the soil installations settled, water and N leaching data began showing treatment trends by spring, 2019.

Leachate NH₄-N concentrations were less across treatments in winter 2018 (not long after drain gauge installation) than they were for summer 2018 (Figure 27).

![NH₄-N Concentration (ppm) over years](image)

**Figure 27.** Season x year interaction ($P < 0.0001; SE = 0.05$) on NH₄-N concentration in leachate of different cropping systems. Average of nine treatments. Summer period refers to the period from Mid-May to October. Winter refers to the period from November of the previous year to early-May.

The summer 2018 elevated NH₄-N concentrations were likely due to effects following soil disturbance from drain gauge installation and the warming soils. A similar pattern was observed for leachate NO₃-N concentrations (Figure 28). However, unlike the NH₄-N values in 2019 and 2020 being similarly low, NO₃-N in summer 2019 was the only sampling period that was lower than summer 2018. Average NO₃-N values remained below 15 ppm across all years. This is less than contributions typically reported from many other cropping systems but there is growing concern that this may not yet be low enough to protect sensitive water bodies, particularly Florida springs.
Figure 28. Season × year interaction ($P = 0.014; \text{SE} = 2.1$) on NO3-N concentration in leachate of different cropping systems. Average of nine treatments. Summer period refers to the period from Mid-May to October. Winter refers to the period from November of the previous year to early-May.

Leachate NO3-N concentrations pooled across years/seasons demonstrated that systems without a cover crop had greater leachate NO3-N than with cover (Figure 29). Note that this includes the summer values when many of the systems included a row crop. As expected, the sod-based system (grazed bahiagrass during winter and summer during this phase of the trial) had among the lowest leachate NO3-N, which typically averaged below 10 ppm (Figure 29). It will be interesting to monitor the impact of converting bahiagrass to the row crop (peanut) on leachate inorganic N during the next phase of this research project. Contributions from decomposing and mineralizing bahiagrass roots/thatch from the SBR and greater soil disturbance with peanut digging across all row crop treatments will likely result in temporary flushes of leachable N.
Figure 29. Contrast effect\(^{(a,b,c)}\) on NO\(_3\)-N concentration in leachate of different cropping systems. Average of three years and two seasons.

\(^{a}\) Contrasts were declared different at the 10% probability level according to the F test. Only significant contrasts are shown in the Figure.

\(^{b}\) No Cover vs. Cover (\(P = 0.06\)); No Grazing vs. Grazing (\(P = 0.15\)); SBR vs. ICL (\(P = 0.09\)); No Grazing vs. ICL (\(P = 0.72\)); No Grazing vs. SBR (\(P = 0.04\)).

\(^{c}\) Cover = Cover 34, Cover 90, SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Cover = No Cover; Grazing = SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Grazing = No Cover, Cover 34, Cover 90; ICL = ICL-Over, ICL-Mod, ICL-Under; SBR = SBR-Over, SBR-Mod, SBR-Under.

Due to high field variability and limited replications, it has been difficult to statistically delineate treatment differences, but some clear patterns in water and nutrient losses were observed over the past year. Primarily, it is evident that the greatest risk for water and nutrient losses in Ag systems of the humid Eastern U.S., is during winter compared to summer seasons (Soldat et al., 2017). This was the case in 2019 and 2020 in our study that demonstrated greater potential N leaching loss in “Winter” compared with “Summer”, while it was similar across seasons in 2018 (Figure 30). The 2021 data was not analyzed since the summer season was not completed prior to this report. Most of our winter rainfall occurs late December through March and this is also when evapotranspiration losses are lowest. Therefore, using vegetation, such as cover crops during winter months rather than bare soil, can help limit nutrient leaching losses.
It is primarily a combination of N inputs (mineral and organic) and water inputs (rain and irrigation events) that drive N leaching losses. Organic N forms eventually mineralize to inorganic N and in North Florida, the majority is oxidized to NO$_3$-N. Different cover crop management configurations tended to impact cumulative NO$_3$-N leaching losses over time. Means contrast results demonstrate relative impacts. To begin, treatments with a cool-season cover crop tended to result in less N leaching loss (Figure 31). Due to field variability, all cover crop N analytic tests were conducted at alpha=0.1, which is typically used to address many biological systems. In comparison, N loss under grazing greatly lessened N leaching loss ($P=0.01$) compared to ungrazed management. Among grazing systems, the sod-based rotation (SBR) tended to have lower N values, but it was not statistically supported. Even so, compared to no grazing, both, SBR and integrated crop=livestock (ICL) led to lower N leaching losses at $P=0.01$ and $P=0.10$, respectively. More specific contrasts are illustrated in Figure 32.
Figure 31. Contrast effect on cumulative total N leaching of different cropping systems. Average of nine treatments. No Cover vs. Cover ($P = 0.10$); No Grazing vs. Grazing ($P = 0.01$); SBR vs. ICL ($P = 0.19$); No Grazing vs. ICL ($P = 0.10$); No Grazing vs. SBR ($P = 0.01$). Cover = Cover 34, Cover 90, SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Cover = No Cover; Grazing = SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Grazing = No Cover, Cover 34, Cover 90; ICL = ICL-Over, ICL-Mod, ICL-Under; SBR = SBR-Over, SBR-Mod, SBR-Under.

The wide range of crop management treatments provided different ways to approach these results. For instance, using a cover in winter will also utilize soil N to support plant growth. Including livestock adds an additional layer of complexity to the system, as plant material (and N) is ingested, but some of that ingested N is returned to the soil through excreta. If livestock are growing (as in this study), then N gets incorporated into animal weight gain. If the system is populated by mature animals, a nutrient steady-state might be reached, where almost all of the ingested N cycles back out via excreta. Since both, SBR and ICL have similar cool-season grazing management, it is understandable that there may not be significant differences on N loss impacts between the two systems. However, the SBR in this study remained in the summer bahiagrass phase during this study period, therefore, it was expected that less seasonal N loss would be expressed. Under moderate grazing management, SBR should result in greater annual root mass than ICL or other systems that are based upon annual plantings. The perennial bahiagrass rhizome are reported to accumulate N in both, aboveground forage and belowground rhizomes (Blue, 1973; Mata and Blue, 1974).
Figure 32. Contrast effect (a, b, c) on cumulative total N leaching of different cropping systems. Average of three years and two seasons.

a Contrasts were declared different at the 10% probability level according to the F test. Only significant contrasts are shown in the Figure.

b No Cover vs. Cover (P = 0.10); No Grazing vs. Grazing (P = 0.01); SBR vs. ICL (P = 0.19); No Grazing vs. ICL (P = 0.10); No Grazing vs. SBR (P = 0.01).

c Cover = Cover 34, Cover 90, SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Cover = No Cover; Grazing = SBR-Over, SBR-Mod, SBR-Under, ICL-Over, ICL-Mod, ICL-Under; No Grazing = No Cover, Cover 34, Cover 90; ICL = ICL-Over, ICL-Mod, ICL-Under; SBR = SBR-Over, SBR-Mod, SBR-Under.
Experiment 2.

In this experiment, we addressed the following topics:

i) Water Footprint, Herbage, and Livestock Responses in N-fertilized and Grass-Legume Grazing Systems

ii) Nitrate leaching from grazing systems with contrasting N inputs

Results and summary of each one of the above topics are detailed below.

i) Water Footprint, Herbage, and Livestock Responses in N-fertilized and Grass-Legume Grazing Systems

Summary: Replacing N fertilizer with forage legumes may increase sustainability of grazing systems. The objectives were to evaluate herbage and animal responses and to quantify the water footprint associated with beef production in N-fertilized grass or grass-legume systems during 4 years under continuous stocking. The three year-round forage systems were: 1) Grass+N which included N-fertilized bahiagrass (*Paspalum notatum* Flüggé) during summer, and it was overseeded with N-fertilized cereal rye (*Secale cereale* L.) and oat (*Avena sativa* L.) during winter; 2) Grass+Clover included bahiagrass without N fertilizer during summer, and it was overseeded with rye, oat, and a mixture of clovers (*Trifolium* spp.) during winter; and 3) Grass+Clover+RP included rhizoma peanut (*Arachis glabrata* Benth.)-bahiagrass mixture during summer, and it was overseeded with a similar rye-oat-clover mixture as for Grass+Clover. Clover inclusion improved uniformity of herbage distribution throughout the winter. Including rhizoma peanut increased cattle average daily gain (ADG) by 74% during summer. The ADG in Grass+Clover+RP was 0.61 kg d\(^{-1}\) compared with 0.35 kg d\(^{-1}\) on Grass+N and Grass+Clover. The water footprint during summer was less in Grass+Clover+RP than Grass+Clover (18 and 25 m\(^3\) kg\(^{-1}\) bodyweight, respectively). Gain per area (GPA) was similar across all treatments through the year, indicating similar productivity in grass-legume and N-fertilized grass systems. The N-fertilizer inputs were reduced from 224 to 34 kg N ha\(^{-1}\) yr\(^{-1}\) in Grass+Clover+RP, compared to Grass+N. Inclusion of rhizoma peanut and clovers contributes to developing sustainable grazing systems with reduced levels of off-farm inputs.

Methods

Experimental Site, Management, and Treatments

A four-year grazing experiment was conducted from January-October of 2016-2019 at University of Florida, North Florida Research and Education Center-Marianna, located in Marianna, FL (30°52’N, 85°11’ W, 35 m A.S.L.). Cool-season months were considered January to early May, while warm-season months were considered late May to October of each year. The 3-yr average number of grazing days were 112 and 165 d in the cool season and warm season, respectively. Soils at the experimental site were classified as Orangeburg loamy sand (fine-loamy-kaolinitic, thermic Typic Kandiudults), with a pH of 5.7. At the beginning of the experiment, average Mehlich-I extractable soil P, K, Mg, and Ca concentrations (from 0 to 15 cm) from air-dried soils were 26, 99, 43, and 224 mg kg\(^{-1}\), respectively. Soil organic matter was 15.4 g kg\(^{-1}\) and estimated cation-exchange capacity was 3.8 meq 100 g\(^{-1}\). Monthly rainfall and average temperatures are shown in Table 18.

Treatments were three grazing systems including warm-season perennial forages in the summer overseeded with cool-season annual species in the winter (Table 19). Treatment Grass+N consisted of N-
fertilized ‘Argentine’ bahiagrass during the summer, overseeded with an N-fertilized mixture of ‘FL401’ cereal rye and ‘RAM’ oat, each planted at a seeding rate of 56 kg ha⁻¹, in the fall for winter forage production. Total annual N fertilization was 224 kg N ha⁻¹ for the Grass+N system, split equally in warm and cool seasons. Treatment Grass+Clover consisted of bahiagrass receiving no N during the summer, that was overseeded in fall with an N-fertilized (34 kg N ha⁻¹) rye-oat-clover mixture, consisting of ‘Dixie’ crimson (Trifolium incarnatum L.), ‘Southern Belle’ red (Trifolium pratense L.), and ball (Trifolium nigrescens Viv.) clovers seeded at rates of 16.8, 6.7, and 3.4 kg ha⁻¹, respectively. Treatment Grass+Clover+RP included Ecoturf rhizoma peanut, strip-planted with bahiagrass (50% of pasture area in each species allocated in 2.7-m wide alternating strips). No N fertilizer was applied to Grass+Clover+RP during the summer, but in fall, pastures were seeded to a similar oat-rye and clover mixture and the winter N-fertilizer scheme was the same as for Grass+Clover. Each pasture (experimental unit) measured 0.85 ha, and treatments were replicated three times in a randomized complete block design. Each experimental unit received the same treatment during all years and there was no reassignment of treatments to experimental units in each year.

All pastures were disked and harrowed prior to planting the perennial species. The rhizoma peanut was strip-planted simultaneously with seeding of bahiagrass on 12 June 2014. Bahiagrass and rhizoma peanut strip width measured 2.7 m each, and in total, each pasture contained 10 strips of both rhizoma peanut and bahiagrass. Bahiagrass was seeded at 28 kg ha⁻¹, and rhizoma peanut was planted at 1000 kg rhizomes ha⁻¹ using a Holden Sodmaster No-Till Bermudagrass Sprigger (Holden-Sodmaker). Each year after the first frost in fall, summer vegetation was mowed at 5-cm stubble height, and cool-season forages were planted using a grain drill (Massey Ferguson MF43). Planting dates for the cool-season forages were 13 Nov. 2015, 2 Dec. 2016, 4 Dec. 2017, and 20 Nov. 2018.

All pastures were fertilized with 34 kg N, 19 kg P, 47 kg K, and 13.4 kg S ha⁻¹ three weeks after cool-season forages were seeded. In April 2016, all pastures were fertilized with 93 kg K, 27 kg Mg, and 12.1 kg S ha⁻¹ using K-Mag (0-22-22-11; Mosaic Co.) and 2.24 kg B ha⁻¹. The Grass+N treatment received 78 kg N ha⁻¹ with 50% as polymer coated urea (ESN) and 50% as urea every year in January. Additionally, Grass+N pastures received 56 kg N ha⁻¹ (46-0-0) in the form of urea in May and July. Imazapic was applied at a rate of 291 mL ha⁻¹ (0.07 kg a.i. ha⁻¹) over the rhizoma peanut strips, in August 2016, May 2017, May 2018, and June 2019, to minimize broadleaf weed pressure.

Pastures were continuously stocked using a variable stocking rate, and put-and-take animals were used to adjust herbage allowance every 14 days (Sollenberger, Moore, Allen, & Pedreira, 2005). Two Angus crossbred steers (Bos spp.) were considered testers and remained on each pasture throughout the experiment each year. Water, shade, and a mineral supplement mixture [Ca = min. 150 and max. 190 g kg⁻¹, P = min. 30 g kg⁻¹, NaCl = min. 150 and max. 180 g kg⁻¹, Mg = min. 100 g kg⁻¹, Zn = min. 2800 mg kg⁻¹, Cu = min. 1200 mg kg⁻¹, I = min 68 mg kg⁻¹, Se = 30 mg kg⁻¹, Vitamin A = 308370 units per kg, Vitamin D3 = 99119 units per kg (Special Mag, W.B. Fleming Company)] were available for cattle in each pasture.

Herbage Responses

Herbage Mass, Allowance, and Accumulation Rate - Cool Season

Herbage mass was quantified every 14 d using the double sampling method (Haydock & Shaw, 1975; Wilm, Costello, & Klipple, 1944). A falling plate meter was used for indirect measures. Thirty disk heights were taken every 14 d at random sites within each pasture. A calibration equation for the disk was developed every 28 d, with the measured herbage mass regressed on the disk height. Each prediction equation had 18 points for grass only and for grass-legume mixtures. At each of the double sampling sites, the forage was clipped at a 5-cm stubble height and dried at 55°C for 72 h to measure actual herbage mass. Across the four years, the R² of the double sampling procedure (avg. ± standard deviation) during the cool season was 0.83 ± 0.09 for Grass+N, and 0.72 ± 0.11 for Grass+Clover and Grass+Clover+RP.
Herbage allowance was estimated every 14 d, with the method described by Sollenberger et al. (2005). Put-and-take animals were used throughout the experimental period to maintain similar herbage allowance among treatments within each block. Target herbage allowance was 1.0 kg DM kg⁻¹ BW during the cool season. Herbage accumulation rate was determined using four exclusion cages per pasture, placed at random sites. Disk height was measured in the previous and new site every 14 d (Vendramini et al., 2012). The same equation developed for herbage mass was used to calculate the pre- and post-herbage mass for each cage site. To calculate the herbage accumulation rate (kg ha⁻¹ d⁻¹), the difference between post- and pre-herbage mass was divided by the number of days the cage was in place (14 d). The total herbage accumulation rate in the pastures with clover was assessed for each component in the sward by multiplying the herbage accumulation rate by the proportion of grass or clover (only in the legume-containing treatments) in each pasture obtained from the botanical composition (% of dry weight).

**Nutritive Value – Cool Season**

Forage hand-plucked samples were collected every 14 d for each functional group (i.e., grass and legume) present in the sward. Samples were dried at 55°C for 72 h and ground to pass a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley laboratory Mill, Thomas Scientific). After grinding the samples, in vitro digestible organic matter (IVDOM) was determined for grass and legume hand-plucked samples using the two-stage technique (Moore & Mott, 1974). Determination of IVDOM concentration was carried out on samples within the first collection of each month. Subsamples were ball-milled in a Mixer Mill MM 400 (Retsch) for 9 min at 25 Hz, and analyzed for N and δ¹⁵N using a CHNS analyzer (Vario Micro Cube, Elementar Inc.) coupled to an isotope ratio mass spectrometer (IsoPrime). Crude protein concentration (CP, g kg⁻¹) was estimated as total N × 6.25.

**Biological N₂ Fixation – Cool Season**

Biological N₂ fixation was determined for the clovers using the natural abundance technique (Freitas, Sampaio, Santos, & Fernandes, 2010). Reference plants (n=5) were collected every 28 d and were classified to the species level, dried at 55°C for 72 h, ground to pass a 2-mm screen, and ball-milled. The proportion of plant N derived from the atmosphere (%Ndfa) was estimated using Eq. [1] described by Shearer and Kohl (1986):

\[
\%N_{\text{dfa}} = \frac{\delta^{15}N_{\text{reference plant}} - \delta^{15}N_{\text{N₂ fixing legume}}}{\delta^{15}N_{\text{reference plant}} - B} \times 100
\]  

where the δ¹⁵Nreference is the δ¹⁵N value for the non-N₂-fixing reference plant, δ¹⁵N N₂-fixing legume is the δ¹⁵N value for the N₂-fixing clover, and B is the δ¹⁵N value for N₂-fixing plant grown in absence of inorganic N. Clover B value (-0.94‰) used in this study was reported by Unkovich et al. (2008). The shoot N accumulation was estimated by multiplying herbage accumulation by legume N concentration. Clover herbage accumulation was estimated by multiplying the proportion of clover in the botanical composition by the herbage accumulation for the given evaluation period. Herbage BNF was estimated by multiplying shoot N accumulation by the %Ndfa. The seasonal BNF was then estimated by multiplying the herbage BNF by the number of days within the cool season for each year. Botanical composition was determined using the dry-weight rank method (Mannetje & Haydock, 1963), in March of each year. In each pasture, 30 random sites were sampled using a 0.25-m² metallic ring. Visual estimation (% of dry-weight, DW) was recorded for all species present and classified as either grass (rye, oat), legume (clovers), or weeds for evaluations during the cool season.

**Herbage Mass, Herbage Allowance, and Herbage Accumulation Rate – Warm Season**

In the warm season, 30 disk heights were taken at random sites within Grass+Clover and Grass+N pastures since these treatments were bahiagrass monocultures in summer. Sixty disk heights per pasture were taken at random sites in the Grass+Clover+RP pastures, 30 points in the strips of each botanical component (bahiagrass or rhizoma peanut). A similar approach to that in the cool season was utilized to estimate herbage mass and herbage allowance (Haydock & Shaw, 1975; Wilm et al., 1944).
During the warm season, the $R^2$ of the equations predicting herbage mass from disk height was $0.80 \pm 0.09$ for Grass+N and Grass+Clover, and $0.76 \pm 0.11$ for Grass+Clover+RP. Target herbage allowance was 1.5 kg DM kg$^{-1}$ BW during the warm season.

*Nutritive Value, Biological N$_2$ Fixation, and Botanical Composition – Warm Season*

Forage nutritive value and BNF were estimated as described for the cool season, with the exception that rhizoma peanut herbage accumulation was estimated by multiplying the proportion of rhizoma peanut in the botanical composition by the herbage accumulation for the given evaluation period. The seasonal BNF was estimated via summation of the evaluation means to obtain a seasonal average. The $B$ value used in this study was -1.41‰, as reported by Okito, Alves, Urquiaga, and Boddey (2004) for *A. hypogea* L. Botanical composition was also determined using the dry-weight rank method (Mannetje & Haydock, 1963), in July of each year, utilizing a 0.25-m$^2$ metallic ring. In the Grass+Clover+RP treatment, 60 random sites (30 within rhizoma peanut strips and 30 within bahiagrass strips) were sampled. In the bahiagrass monoculture pastures (Grass+Clover, Grass+N), 30 random sites were sampled to estimate the percentage of grass, legume, and weeds.

*Animal Performance*

**Average daily gain, gain per area, and stocking rate**

The methodologies to assess animal performance were similar for both cool and warm seasons. The body weight (BW) of the tester steers was measured every 21 d after 16 h withdrawal from feed and water. Initial BW of tester steers was 224 ± 27, 311 ± 31, 277 ± 17, and 230 ± 29, for 2016, 2017, 2018, and 2019, respectively. The same animals remained on their corresponding pastures during the 9 months of grazing each year, encompassing both the cool and warm seasons. Average daily gain was calculated for each 21-d period by dividing the average weight gain of the two testers per pasture by the number of days (kg hd$^{-1}$ d$^{-1}$). The ADG over the entire year (cool + warm season) was estimated as a weighted average based on ADG per given season and year and the length of the season per given year. Grazing days were calculated by multiplying the total number of animal units (AU, 350 kg BW) in each pasture (both tester and put-and-take) by the number of days within each period, and subsequently summing all the animal days at the end of each season. Gain per area (kg ha$^{-1}$) was calculated by multiplying ADG by the number of grazing days per hectare within each period. Stocking rate was calculated by dividing the grazing days by the total number of days within each season.

*Proportion of rhizoma peanut in the diet*

The proportion of rhizoma peanut in the diet was estimated using the $\delta^{13}$C from feces and plant material (Jones, Ludlow, Troughton, & Blunt, 1979). Fecal grab samples were collected at each weighing period during the warm season (every 21 d), when the animals were brought to the weighing facilities to begin the feed and water withdrawal period. Fecal samples were collected directly from the rectum and placed in quart-sized plastic bags. The feces were frozen at -20°C, until they could be processed. All fecal samples were thawed, dried at 55°C until they reached a constant weight, and ground to pass a 2-mm stainless steel screen using a Wiley Mill. Subsequent $\delta^{13}$C analysis followed the same procedure as the forage hand-plucked samples. The proportion of rhizoma peanut was estimated using Eq. [2] described by Jones et al. (1979):

$$\%RP = 100 - \left\{100 \times \frac{A-C}{B-C}\right\}$$  \[2\]

where $\%RP$ is the proportion of rhizoma peanut in the feces, $A$ is the $\delta^{13}$C of the fecal sample obtained, $B$ is the $\delta^{13}$C of bahiagrass, and $C$ is the $\delta^{13}$C of rhizoma peanut. The $\delta^{13}$C from the closest evaluation date to the fecal sampling was utilized for both bahiagrass and rhizoma peanut. The selection index was calculated by dividing the $\%RP$ estimated from the feces and the proportion of rhizoma peanut in each pasture based on botanical composition.

*System Water Footprint*
The water footprint evaluations were limited to the warm seasons (May to Oct.) in 2018 and 2019. Each pasture contained an individual water trough (415-L capacity), coupled to a water meter (Hersey-Meters Model No. VOGA204). Each water trough contained an automatic shut-off valve, sustaining 300-L base volume across all water troughs utilized. Water meter readings were recorded every 21 d. Pastures did not receive supplemental irrigation, and all systems were considered rain fed. Rainfall was tracked utilizing a rain gauge (Onset Computer Corporation, RG3-M) at the experimental site.

Water consumption by livestock was estimated by subtracting pre- and post- water meter readings on water troughs for each period. Spillage from the animal’s drinking process was integrated in the drinking water intake. The rainfall volumes were estimated by multiplying the rainfall depth by the area of each pasture. Similarly, the rainfall volumes into each water trough was also estimated using the surface area of the water trough (0.86 m²), since they were open troughs. During periods when there was water trough overflow, the average water volume was used from previous and post evaluations to avoid missing data points. There were three missing evaluations in 2018 and two in 2019. The yearly water budgets were calculated by summing the rainfall in each pasture + rainfall added to each water trough, as well as water consumption from water meters. The water footprint was estimated in two manners, one of which accounted for rainfall and drinking water and the other based on drinking water only. In both cases, the water footprint was expressed on BW gain per area, in m³ kg⁻¹ BW gain. Water consumption rate by individual animals was estimated by dividing the amount of water consumed, according to the water meters, by the stocking rates over each 21-d period. The water footprint, based on rainfall and drinking water, was calculated in Eq. [3]:

\[ \text{WF} = \frac{\text{rainfall+ drinking water}}{\text{GPA}} \]  

where WF is the water footprint and GPA is the gain per area, expressed in kg BW gain. In addition, the water footprint limited to blue water, was calculated using Eq. [4]:

\[ \text{WF} = \frac{\text{drinking water}}{\text{GPA}} \]

For this study drinking water was considered blue water. Blue water refers to water stored in water bodies (Ran et al., 2016), which in this study represents the water usage diverted from a well.

Statistical Analyses

All response variables were analyzed using linear mixed model procedures as implemented in SAS PROC GLIMMIX (SAS/STAT 15.1; SAS Institute). Pastures were considered experimental units for all output variables. For responses including ADG, gain per area, stocking rate, and water footprint, the model included treatment, evaluation period, and their interaction as fixed effects. Block, year, and block × treatment were considered random effects. All other herbage (herbage mass, herbage accumulation, nutritive value, isotopic composition, selection index, and BNF) responses were repeated measures. The covariance function resulting in the lowest AICC fit statistic was selected for each response variable. Least squares treatment means were compared through pairwise t-test using the PDIFF option of the LSMEANS statement in the aforementioned procedure. Based on the recommendations by Milliken and Johnson (2009) and (Saville, 2015), no adjustment for multiple comparisons was made. Differences were considered significant at \( P \leq .05 \). An exception was made for water footprint responses, where significance was declared at \( P \leq .10 \).

Results

Herbage Responses – Cool Season

Herbage accumulation rates during the cool season showed a treatment × evaluation date interaction (\( P = .01 \)). Grass+N peaked earlier in the season, in early March, than Grass+Clover+RP and Grass+Clover. Grass+N also showed a second peak after N-fertilizer application in early April (Fig. 33A). Herbage accumulation rate in Grass+N was 40 kg DM ha⁻¹ d⁻¹ at both peaks and declined after early April. Grass+Clover (70 kg DM ha⁻¹ d⁻¹), and Grass+Clover+RP (50 kg DM ha⁻¹ d⁻¹) had their greatest herbage accumulation rates in late April.
As expected, similar pattern was observed in herbage mass over time, where there was also a treatment × evaluation date interaction ($P = .03$; Fig. 33B). However, there was no treatment difference in herbage mass among the three treatments ($P = .75$), where on average, there was 700 kg DM ha$^{-1}$ in each system. Additionally, BNF in the Grass+Clover and Grass+Clover+RP treatments during the cool season (January to early May), did not differ ($P = .66$), with BNF average of 41 kg N ha$^{-1}$ season$^{-1}$.

Crude protein concentrations in the grass component did not differ among treatments ($P = .23$) but differed across evaluation dates through the cool season ($P < .001$; Fig. 34C). The greatest grass CP concentrations were observed in early and late January, with values of 251 g kg$^{-1}$. Clover CP concentrations (Grass+Clover and Grass+Clover+RP) did not differ between treatments ($P = .22$), but they differed across evaluation dates ($P < .001$), with least CP concentrations observed in early May (208 g kg$^{-1}$) and greatest in late March (271 g kg$^{-1}$; Fig. 34A). Clover IVDOM concentration also differed among evaluation dates ($P < .001$) as well and were above 750 g kg$^{-1}$ from January through March, decreasing to 675 g kg$^{-1}$ by the end of the cool season (Fig. 34B). Grass IVDOM concentrations also differed by evaluation dates, having similar concentrations in January and February (~750 g kg$^{-1}$), decreasing to 650 g kg$^{-1}$ by April (Fig. 34D).

**Herbage Responses – Warm Season**

Herbage accumulation rate was affected by a treatment × evaluation date interaction ($P = .01$; Fig. 35A). Throughout most of the evaluation dates, Grass+Clover+RP had the least herbage accumulation rates, and on average, this treatment produced 24 kg DM ha$^{-1}$ d$^{-1}$. Grass+N had greater rates of herbage accumulation from May through late July, where it remained constant, around 60 kg DM ha$^{-1}$ d$^{-1}$. Grass+Clover pastures, lacking N-fertilizer application during the warm season, showed lesser herbage accumulation rates the first half of the season but then peaked in late August (80 kg DM ha$^{-1}$ d$^{-1}$) before decreasing thereafter. Similarly, herbage mass also showed a treatment × evaluation date interaction ($P = .007$; Fig. 35B). Grass+N and Grass+Clover had consistently greater herbage mass than Grass+Clover+RP ($P = .002$). The least herbage mass was observed across the three treatments during the beginning of the warm season (late May - late June), averaging 850 kg DM ha$^{-1}$. The rhizoma peanut composed an average of 32% in proportion of the pasture botanical composition, and its BNF averaged 16 kg N ha$^{-1}$ season$^{-1}$, from 2016-2019 (Table 20).

Grass CP concentration differed among treatments ($P = .002$), where Grass+N had the greatest average CP concentration (120 g kg$^{-1}$), and compared with Grass+Clover and Grass+Clover+RP treatments at 111 g kg$^{-1}$. There was also an evaluation date effect ($P < .001$; Fig. 36A) on the grass CP concentration. Overall, there was a decreasing trend in CP from May through October, in which they were greatest in early June and least by early October (162 and 75 g kg$^{-1}$, respectively). There was also an evaluation date effect ($P < .001$; Fig. 36B) on grass IVDOM concentration, where IVDOM was above 480 g kg$^{-1}$ from May through July. By September, the bahiagrass IVDOM concentration reached its minimum at 380 g kg$^{-1}$. For the rhizoma peanut component of Grass+Clover+RP pastures, the CP and IVDOM concentrations did not differ along the evaluation dates ($P = .13$, SEM= 10 and 0.79, respectively), and averaged 187 g CP kg$^{-1}$ DM, and 668 g DOM kg$^{-1}$ OM, respectively. Bahiagrass composed 92, 82, and 47% of the total botanical composition in Grass+N, Grass+Clover, and Grass+Clover+RP, respectively ($P < .001$), while in Grass+Clover+RP, the rhizoma peanut composed 32% of the total botanical composition.

**Animal performance**

**Cool season**

Average daily gain did not differ ($P = .47$) among treatments during the cool season across the 4 years (Table 20), and averaged 0.85 kg d$^{-1}$. Additionally, gain per area during the cool season did not differ among the three treatments ($P = .90$) and averaged 282 kg BW ha$^{-1}$. Stocking rates did not differ...
by treatment ($P = .59$) and were 2.9 AU ha$^{-1}$ across treatments. Herbage allowance did not differ among treatments during the cool season ($P = .73$), averaging 0.7 kg DM kg$^{-1}$ BW.

**Warm season**

Average daily gain during the warm season differed across treatments ($P = .01$), with Grass+Clover+RP having greater ADG (0.61 kg d$^{-1}$) than Grass+N and Grass+Clover (0.34 kg d$^{-1}$). Within Grass+Clover+RP, there was no effect of sampling date ($P = .11$) on proportion of RP in the diet (44%), nor on the selection index ($P = .27$), which averaged 1.38 across the warm-season evaluation periods. Gain per area during the warm season was also greatest in Grass+Clover+RP ($P = .04$; 397 kg ha$^{-1}$), while Grass+Clover was least with 278 kg ha$^{-1}$. There were treatment differences in stocking rate ($P = .003$), with Grass+N supporting the greatest stocking rates, Grass+Clover+RP the least, and Grass+Clover an intermediate level (6.3, 5.6, and 4.3 AU ha$^{-1}$, respectively). Herbage allowance did not differ among all treatments during the warm season ($P = .96$) and averaged 1.2 kg DM kg$^{-1}$ BW.

**Warm + Cool Seasons**

The annual (cool + warm season) ADG did not differ among treatments ($P = .18$), where the ADG was 0.61 kg d$^{-1}$. Gain per unit land area ($P = .46$) also did not differ among treatments and averaged 618 kg BW ha$^{-1}$ (Table 21). Stocking rates were greater for Grass+N and Grass+Clover throughout the year ($P = .01$) averaging 4.3 AU ha$^{-1}$, while stocking rate for Grass+Clover+RP was least (3.7 AU ha$^{-1}$).

**Warm-Season Beef Water Footprint and Water Intake**

The water footprint of the forage systems differed across treatments ($P = .07$), when considering rainfall and drinking water (Table 22). The water footprint was 18 m$^3$ kg$^{-1}$ BW gain for Grass+Clover+RP, compared with 20 and 25 m$^3$ kg$^{-1}$ BW gain in Grass+N and Grass+Clover, respectively, when considering rainfall and drinking water. When considering only drinking water, the water footprint was the least in Grass+Clover+RP (0.06 m$^3$ kg$^{-1}$ BW gain), while Grass+N and Grass+Clover were similarly greater (0.11 m$^3$ kg$^{-1}$ BW gain, each). The overall water consumption per animal did not differ across treatments and averaged 43 L hd$^{-1}$ d$^{-1}$.

**Discussion**

**Herbage Responses – Cool Season**

Mixing forage species with complementary seasonality of plant growth is an effective strategy for ensuring adequate forage mass throughout an extended grazing season (McCormick, Sulc, Barker, & Beuerlein, 2006). In this study, herbage accumulation rate and herbage mass differed based on the sward components during the cool season. Small grains provide earlier growth, compared with clovers (Dubeux et al., 2016), and N fertilization resulted in greater herbage mass and accumulation rate of Grass+N earlier in the cool season. Grass+N had greater herbage mass earlier in the cool season as a result of greater N-fertilizer applications. Herbage accumulation rate and herbage mass began to decline in early March for Grass+N, at which time Grass+Clover and Grass+Clover+RP productivity increased, due to increasing clover productivity. Thus, the benefits of including clovers are highlighted, especially since herbage mass was sustained for Grass+Clover and Grass+Clover+RP pastures well into late spring, while Grass+N (no legume) was in decline. Fontaneli, Sollenberger, and Staples (2000) also reported similar results, where clover inclusion in rye-oat pastures extended the grazing season in North Florida. Overall, this corroborates findings by others (Butler, Biermacher, Kering, & Interrante, 2012; Sanderson, Brink, Stout, & Ruth, 2013) who reported grass-legume mixtures can produce as much biomass as N-fertilized grass monocultures, and that legume inclusion into grass monocultures improves forage seasonal distribution (Fontaneli et al., 2000; Sleugh, Moore, George, & Brummer, 2000).

Biological N$_2$ fixation is an important ecosystem service obtained through inclusion of clovers in cool-season pastures. Clover in the southeastern U.S. is reported to fix up to 155 kg N ha$^{-1}$ yr$^{-1}$ in pure stands (Brink, 1990). The BNF values observed in this study are below these estimates, since grazing
affects clover biomass, and the overall BNF contribution is a function of legume proportion in the mixture, herbage accumulation, N concentration, and %Ndfa.

Despite Grass+N receiving an additional 78 kg N ha⁻¹ fertilizer during the cool season, compared with Grass+Clover and Grass+Clover+RP, the CP and IVDOM concentrations of the grass components did not differ. The CP concentrations were similar to those reported by Dubeux et al. (2016) for N-fertilized rye-ryegrass mixed pastures under grazing management. Grasses in the grass-legume mixtures apparently benefitted from N transfer (whether direct or indirect) from legume species (Dubeux et al., 2007). Although not measured in this study, direct and indirect N transfer from clover to the cool-season grasses via nutrient cycling are likely involved. The decline in CP concentration observed over time is attributed to maturity, stem elongation, and flowering of small grain components in all treatments (Butler et al., 2012). Crude protein, from both grasses and clovers, coupled with excellent IVDOM concentrations, often exceeded those established by the National Academies of Sciences & Medicine for meeting the nutritional requirements for several classes of beef cattle (National Academies of Sciences & Medicine, 2016).

**Herbage Responses – Warm Season**

Although rhizoma peanut composed only 32% of the total botanical composition in the Grass+Clover+RP treatment, it composed 45% of the cattle diet, suggesting cattle preference for rhizoma peanut over the bahiagrass companion. In addition, the selection index greater than 1.0 is further evidence of livestock preference for rhizoma peanut, over bahiagrass, throughout the warm season. This preference likely contributed to less than expected herbage mass for this treatment. The seasonal herbage mass for Grass+Clover+RP followed a similar growth distribution to what has been reported for bahiagrass grown in Florida (Beaty, Powell, Brown, & Ethredge, 1963; Gates et al., 2001; Vendramini et al., 2013).

The rhizoma peanut BNF was less compared with studies evaluating tropical grass-legumes mixtures under grazing. For instance, Thomas, Asakawa, Rondon, and Alarcon (1997) reported up to 40 kg N ha⁻¹ in grazed pastures containing *Urochloa dictyoneura* (Fig. and De Not.) Veldkamp and one of three legumes, *A. pintoi* Krapov. & W.C. Gregory, *Centrosema acutifolium* Benth, or *Stylosanthes capitata* Vogel. However, in pure stands and under hay production management, rhizoma peanut cultivars may fix 200 kg N ha⁻¹ yr⁻¹ (Dubeux et al., 2017). In mixed grazed pastures, BNF is a function of legume proportion in the herbage mass, legume herbage accumulation, persistence, and competition from other species, among others (Ledgard & Steele, 1992). The strip-planting arrangement and management of this treatment may have enabled selection of rhizoma peanut by cattle, resulting in somewhat suppressed herbage accumulation rates and lesser overall contribution via BNF.

Nitrogen fertilization management differences did not affect bahiagrass CP or IVDOM concentrations during the warm season. However, declining in CP and IVDOM concentrations observed during the growing season are common in bahiagrass pastures (Stewart Jr. et al., 2007).

**Animal Responses**

**Cool season**

Animal gains during the cool season were similar to those reported by Dubeux et al. (2016) for steers grazing various cool-season forage mixtures. The benefit of clover inclusion was also evident, especially since gains did not differ among treatments, despite clover treatments receiving 78 kg ha⁻¹ less N fertilizer during the cool season. Although stocking rates were greater in Grass+N, the GPA was similar across all treatments during the cool season, which indicates the value of clover inclusion into grazing systems. Nitrogen fertilizer is an important input affecting profitability of beef cattle operations. Production costs can be reduced through utilizing clovers since N fertilizer costs may be greater than clover seed (Butler et al., 2012).
Warm season

Nitrogen fertilization did not improve steer performance during the warm season, while ADG values were similar to what has been reported for continuously stocked bahiagrass pastures (Sollenberger, Rusland, Jones Jr, Albrecht, & Gieger, 1989; Stewart Jr. et al., 2007). In contrast, inclusion of rhizoma peanut (Grass+Clover+RP), improved ADG and GPA. Adoption of grass-legume systems may be beneficial for meeting nutritional demands of livestock having greater requirements, such as lactating beef cows, while reducing potential supplementation costs (National Academies of Sciences & Medicine, 2016).

In addition, strip-planting rhizoma peanut into bahiagrass swards was shown to improve livestock performance while reducing N fertilizer inputs. Livestock gains during the warm season in the southeastern U.S. tend to decrease as the season progresses, in part, given the lesser nutritive value of subtropical perennial grasses. Therefore, it greatly benefits the producer to have a sustainable option for addressing this problem.

Cool + warm season

On an annual basis, legume inclusion into grazing systems resulted in similar livestock ADG and GPA as systems relying on N fertilizers, and the main advantage for Grass+N was greater stocking rates. Producers relying on N-fertilized grass systems must compare economic returns considering greater fertilizer but also greater herbage accumulation and stocking rates of these vs. grass-legume systems. Oftentimes, GPA is considered the variable of greatest economic importance, integrating both stocking rate and animal performance (Sollenberger & Cherney, 1995). Comparable ADG and GPA for Grass+Clover+RP and Grass+N indicates that forage legumes may be beneficial from environmental (reduced N losses) and economic (reduced input costs) perspectives.

On an annual basis, N-fertilizer inputs were reduced from 224 (Grass+N) to 34 kg N ha⁻¹ yr⁻¹ (Grass+Clover+RP) yet animal performance was sustained or sometimes increased. Management aimed at reducing N-fertilizer inputs will continue to play an important role in the sustainability of livestock production. Reducing N-fertilizer inputs contributes to diminishing the agricultural C footprint (Lal, 2004) and potential for environmental pollution issues from beef production systems. In several instances, N fertilizer use has contributed to increased nitrate leaching into groundwater and surface waters (Owens & Bonta, 2004), as well as emissions of nitrogenous greenhouse gases from soil (Fenn et al., 1998).

Implementing grass-legume mixtures in pasture systems has the potential to reduce off-farm inputs while sustaining desirable livestock performance.

Water Footprint of Beef Production

The positive effects of rhizoma peanut inclusion were reflected in the water footprint estimates. The greater animal performance during the warm season in Grass+Clover+RP was a result of greater forage nutritive value from the rhizoma peanut component in the pasture. As a whole, the greater GPA in Grass+Clover+RP was the main driver for decreasing the water footprint, especially in relation to Grass+Clover, which consisted of non-N-fertilized bahiagrass during the warm season. In relation to the water footprint of the drinking water, the combined effects of greater ADG and decreased stocking rates in Grass+Clover+RP resulted in decreased drinking water volume and overall decreased water footprint, in comparison to the other treatments.

There is no known reporting of water footprints for grazing systems, using direct measurements, as was done in this study. Baxter et al. (2017) reported the water footprint from grazing beef cattle in the Southern Great Plains Region in Texas, USA. Based upon rainfall, irrigation, and drinking water, the average across 3 yr was 34 m³ kg⁻¹ BW gain. Comparisons across these studies are difficult due to differences in approach, calculations and models, along with management assumptions (Ran et al., 2016). However, the data within this study corroborate findings by Baxter et al. (2017) where the inclusion of legumes in grazed pastures resulted in a reduced water footprint in relation to grass-only pastures, especially as a result of improved forage nutritive value from legume inclusion.
The water consumption estimated in this experiment falls within reported ranges (40 – 56 L hd⁻¹ d⁻¹) for growing steers, heifers, and bulls of around 364 kg BW, in regions with ambient temperatures between 26.6, and 32.2°C (National Academies of Sciences & Medicine, 2016; Winchester & Morris, 1956). Water is an essential nutrient for beef cattle, and therefore assessing their water intake is important for potential management decisions (National Academies of Sciences & Medicine, 2016). Overall, a number of factors can influence water intake in grazing beef cattle, however environmental factors, including ambient temperature, wind speed, and humidity are major drivers of water consumption rates (National Academies of Sciences & Medicine, 2016).

**Conclusions**

The inclusion of clovers into cool-season grass pastures improved the forage distribution throughout the grazing season and sustained nutritive value similar to that of N-fertilized grass. Inclusion of the warm-season legume rhizoma peanut increased steer ADG by 74% compared with systems relying on bahiagrass monocultures, even with N fertilization. Nitrogen fertilizer inputs were reduced from 224 (Grass+N) to 34 kg N ha⁻¹ yr⁻¹ (Grass+Clover+RP) while sustaining desirable animal performance. Reducing N-fertilizer inputs not only reduces C footprint of legume-based systems but can also reduce nitrate leaching and greenhouse gas emissions. Warm-season legume inclusion in Grass+Clover+RP was also a key driver to reducing the water footprint of these production systems. Overall, these data indicate that year-round grazing systems including clovers during the cool season and rhizoma peanut during the warm season, have equal or greater performance to N-fertilized grass systems and can potentially increase economic returns since less money is spent on N-fertilizer inputs. Further economic analyses should be conducted to evaluate whether N fertilization can be economically viable in comparison to costs related with legume establishment. Overall, inclusion of rhizoma peanut and clovers contributes to developing sustainable grazing practices with reduced off-farm inputs.
Table 18. Monthly weather data and 30-yr average during the experimental period at University of Florida, North Florida Research and Education Center, Marianna, Florida.

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Table 19. Grazing systems during cool and warm seasons

<table>
<thead>
<tr>
<th>Grass</th>
<th>Legume</th>
<th>Cool season</th>
<th>Warm season</th>
<th>Cool season</th>
<th>Warm season</th>
<th>Cool season</th>
<th>Warm season</th>
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</thead>
<tbody>
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<td>Grass+N</td>
<td>Oat, Rye</td>
<td>112</td>
<td>112</td>
<td>34</td>
<td>-</td>
<td>34</td>
<td>-</td>
</tr>
<tr>
<td>Grass+Clover</td>
<td>Clovers</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Grass+Clover+RP</td>
<td>Bahiagrass</td>
<td>Oat, Rye</td>
<td>Clovers</td>
<td>Oat, Rye</td>
<td>Clovers</td>
<td>Bahiagrass</td>
<td>Rhizoma peanut</td>
</tr>
<tr>
<td>kg N ha⁻¹</td>
<td>112</td>
<td>112</td>
<td>34</td>
<td>-</td>
<td>34</td>
<td>-</td>
<td></td>
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</tbody>
</table>
Table 20. Botanical composition (BC) during the warm season, % of rhizoma peanut (RP) in feces, rhizoma peanut selection index, and biological N$_2$-fixation (BNF) during cool and warm seasons in three grazing systems from 2016-2019. Means are averages of values throughout each year.

<table>
<thead>
<tr>
<th>Grazing System$^a$</th>
<th>Grass+Clover</th>
<th>Grass+N</th>
<th>Grass+Clover+RP</th>
<th>SEM</th>
<th>$P$</th>
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</thead>
<tbody>
<tr>
<td>Grass in BC warm season, %</td>
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<td>92</td>
<td>47</td>
<td>4.1</td>
<td>&lt; .001$^b$</td>
</tr>
<tr>
<td>RP in BC, %</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td>1.9</td>
<td>&lt; .001$^c$</td>
</tr>
<tr>
<td>%RP in feces</td>
<td>-</td>
<td>-</td>
<td>44</td>
<td>10.4</td>
<td>.11$^c$</td>
</tr>
<tr>
<td>Selection index$^d$</td>
<td>-</td>
<td>-</td>
<td>1.38</td>
<td>0.79</td>
<td>.27</td>
</tr>
<tr>
<td>BNF, Cool Season, kg N ha$^{-1}$ season$^{-1}$</td>
<td>44</td>
<td>-</td>
<td>38</td>
<td>20.38</td>
<td>.66$^b$</td>
</tr>
<tr>
<td>BNF, Warm Season, kg N ha$^{-1}$ season$^{-1}$</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>0.83</td>
<td>.03$^c$</td>
</tr>
</tbody>
</table>

$^a$Grass+N = N-fertilized bahiagrass during the warm season, overseeded with cereal rye and oat during the cool season; Grass+Clover = bahiagrass during the warm season, overseeded with rye-oat plus a mixture of clovers during the cool season; Grass+Clover+RP = rhizoma peanut and bahiagrass during the warm season, overseeded with rye-oat-clover mixture during the cool season.

$^b$P-value of treatment effect

$^c$P-value of evaluation date effect

$^d$Selection index, % rhizoma peanut in feces divided by % rhizoma peanut in pasture canopy botanical composition
Table 21. Average daily gain (ADG), gain per area (GPA), stocking rate (AU ha⁻¹), and herbage allowance (kg DM kg⁻¹ BW) in Grass+Clover, Grass+N, and Grass+Clover+RP pastures during cool and warm seasons from 2016-2019, and the whole-year average

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grass+Clover</th>
<th>Grass+N</th>
<th>Grass+Clover+RP</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cool Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg hd⁻¹ d⁻¹</td>
<td>0.91</td>
<td>0.83</td>
<td>0.80</td>
<td>0.08</td>
<td>.47</td>
</tr>
<tr>
<td>GPA, kg ha⁻¹</td>
<td>288</td>
<td>285</td>
<td>273</td>
<td>39.5</td>
<td>.90</td>
</tr>
<tr>
<td>Stocking rate, AU b</td>
<td>2.8</td>
<td>3.0</td>
<td>2.9</td>
<td>0.30</td>
<td>.59</td>
</tr>
<tr>
<td>Herbage allowance, kg DM kg⁻¹ BW</td>
<td>0.78</td>
<td>0.79</td>
<td>0.77</td>
<td>0.10</td>
<td>.73</td>
</tr>
<tr>
<td>Warm Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg hd⁻¹ d⁻¹</td>
<td>0.33 B</td>
<td>0.36 B</td>
<td>0.61 A</td>
<td>0.05</td>
<td>.01</td>
</tr>
<tr>
<td>GPA, kg ha⁻¹</td>
<td>278 B</td>
<td>335 AB</td>
<td>397 A</td>
<td>67.8</td>
<td>.04</td>
</tr>
<tr>
<td>Stocking rate, AU ha⁻¹</td>
<td>5.6 B</td>
<td>6.3 A</td>
<td>4.3 C</td>
<td>0.48</td>
<td>.003</td>
</tr>
<tr>
<td>Herbage allowance, kg DM kg⁻¹ BW</td>
<td>1.22</td>
<td>1.24</td>
<td>1.21</td>
<td>0.06</td>
<td>.96</td>
</tr>
<tr>
<td>Cool + Warm Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg hd⁻¹ d⁻¹</td>
<td>0.56</td>
<td>0.58</td>
<td>0.69</td>
<td>0.07</td>
<td>.18</td>
</tr>
<tr>
<td>GPA, kg ha⁻¹</td>
<td>565</td>
<td>620</td>
<td>669</td>
<td>103</td>
<td>.46</td>
</tr>
<tr>
<td>Stocking rate, AU ha⁻¹</td>
<td>4.4 A</td>
<td>4.9 A</td>
<td>3.7 B</td>
<td>0.34</td>
<td>.01</td>
</tr>
</tbody>
</table>

aGrass+N = N-fertilized bahiagrass during the warm season, overseeded with cereal rye and oat during the cool season; Grass+clover = bahiagrass during the warm season, overseeded with rye-oat plus a mixture of clovers during the cool season; Grass+Clover+RP = rhizoma peanut and bahiagrass during the warm season, overseeded with rye-oat-clover mixture during the cool season.

bAU (animal unit); 1 AU = 350 kg BW.

cMeans followed by the same letter do not differ (P >.05) according to least significant difference. hd, head; DM, dry matter; BW, body weight.
Table 22. Water footprint of beef production (WF; m³ kg⁻¹ BW), total drinking water per pasture and water intake by beef cattle (L ha⁻¹ d⁻¹) during the warm season in 2018 and 2019 in three grazing systems

<table>
<thead>
<tr>
<th>Grazing Systema</th>
<th>Grass+Clover</th>
<th>Grass+N</th>
<th>Grass+Clover+RP</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WF, (rainfall + drinking water, m³ kg⁻¹ BW gain)</td>
<td>25 A</td>
<td>20 AB</td>
<td>18 B</td>
<td>7.2</td>
<td>.07</td>
</tr>
<tr>
<td>WF, drinking water, m³ kg⁻¹ BWc gain</td>
<td>0.11 B</td>
<td>0.11 B</td>
<td>0.06 A</td>
<td>0.035</td>
<td>.04</td>
</tr>
<tr>
<td>Drinking water, m³ ha⁻¹</td>
<td>25 A</td>
<td>26 A</td>
<td>21 B</td>
<td>2.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Water intake, L hd d⁻¹</td>
<td>42</td>
<td>43</td>
<td>44</td>
<td>5.3</td>
<td>.84</td>
</tr>
</tbody>
</table>

aGrass+N = N-fertilized bahiagrass during the warm season, overseeded with cereal rye and oat during the cool season; Grass+clover = bahiagrass during the warm season, overseeded with rye-oat plus a mixture of clovers during the cool season; Grass+Clover+RP = rhizoma peanut and bahiagrass during the warm season, overseeded with rye-oat-clover mixture during the cool season.

bMeans followed by the same letter do not differ (P >.05) according to LSD.

cBW, body weight.
Figure 33. Treatment × evaluation date interactions (P = .01) for total herbage accumulation rate (kg DM ha⁻¹ d⁻¹; A) and herbage mass (P = .03; kg DM ha⁻¹; B) during the cool seasons (January-May) of 2016-2019. Treatments were: Grass+N = N-fertilized bahiagrass during the warm season, overseeded with cereal rye and oat during the cool season; Grass+Clover = bahiagrass during the warm season, overseeded with rye-oat plus a mixture of clovers during the cool season; Grass+Clover+RP = rhizoma peanut and bahiagrass pastures during the warm season, overseeded with rye-oat-clover mixture during the cool season. Evaluation numbers indicate evaluation date, and are as follows: 1- early Jan.; 2- late Jan.; 3- early Feb.; 4- late Feb.; 5- early March; 6- late March; 7- early April; 8- late April; 9- early May. Error bars denote standard errors; * Indicates significant differences within evaluations (P ≤ 0.05) according to LSD; DM, dry matter.
Figure 34. Evaluation date effects on clover crude protein (P < .001; A) and in vitro digestible OM (IVDOM) concentrations (P < .001; B), and grass crude protein (P < 0.001; C) and IVDOM concentrations (P < .001; D). Means are averages during the cool season (January-May) of 2016-2018. Means followed by the same letter do not differ (P >.05) according to LSD. Error bars denote standard error. Evaluation numbers indicate evaluation month, and are as follows: 1- early Jan.; 2- late Jan.; 3- early Feb.; 4- late Feb.; 5- early March; 6- late March; 7- early April; 8- late April; 9- early May.
Figure 35. Treatment × evaluation date interactions ($P = .01$) on herbage accumulation rate (kg DM ha$^{-1}$ d$^{-1}$; A) and herbage mass ($P = .007$; kg DM ha$^{-1}$; B) during the warm seasons (May-October) of 2016-2019. Treatments consist of Grass+N = N-fertilized bahiagrass during the warm season, overseeded with cereal rye and oat during the cool season; Grass+Clover = bahiagrass during the warm season, overseeded with rye-oat plus a mixture of clovers during the cool season; Grass+Clover+RP = rhizoma peanut and bahiagrass during the warm season, overseeded with rye-oat-clover mixture during the cool season. Evaluation numbers indicate evaluation date, and are as follows: 10- Late May; 11- Early June; 12- Late June; 13- Early July; 14- Late July; 15- Early August; 16- Late August; 17- Early September; 18- Late September; 19- Early October; 20- Late October. Error bars denote standard errors; * Indicates significant differences within evaluations ($P \leq 0.05$) according to LSD. DM, dry matter.
Figure 36. Evaluation date effect ($P < .001$) on grass crude protein concentrations (g kg$^{-1}$; A), and evaluation effect on grass ($P < .001$) in vitro digestible organic matter (IVDOM) concentrations (g kg$^{-1}$; B). Evaluation numbers (on A) indicate evaluation date, and are as follows: 10- Late May; 11- Early June; 12- Late June; 13- Early July; 14- Late July; 15- Early August; 16- Late August; 17- Early September; 18- Late September; 19- Early October; 20- Late October. Error bars denote standard errors; Means followed by the same letter do not differ ($P > .05$) according to LSD.
ii) Nitrate leaching from grazing systems with contrasting N inputs

Materials and Methods:

Location: The study was on an established pastures adjacent to the demonstration SBR system at the North Florida Research and Education Center (NFREC), Marianna (30°52’ N, 85°11’ W, 35 m altitude). The soils at the experimental site are Ultisols, Red Bay fine sandy loam and Orangeburg loamy sand. These soils are representative of the Jackson Blue Spring and Chipola River Basins.

Procedures: We substituted N fertilizer with biological N₂ fixing forage legumes and assess the effect on nitrate leaching and livestock productivity. Paddocks contained nine drain lysimeters (one per experimental unit; and replicated three times) and 18 porous cup lysimeters (two per experimental unit and replicated three times). Treatments were assigned in a complete randomized block design, with three replications.

Grazing system treatments:

i) Rye/oats + 100 lb. N/acre (similar to SBR) during the cool-season and 100 lb. N/acre during the warm-season on bahiagrass.

ii) Rye/Oat/Crimson/Red/Ball clover + 30 lb. N/acre during the cool-season and unfertilized bahiagrass during the warm-season.

iii) Rye/Oat/Crimson/Red/Ball clover + 30 lb. N/acre during the cool-season and unfertilized bahiagrass/perennial peanut during the warm-season. Cool-season forages will be no-till drilled onto dormant bahiagrass sod during the fall. Seeding rates for rye and oat are similar to the ones used in the first experiment, i.e., 50 lbs of seed/acre of each component (100 lbs/acre of the mix). Clover seeding rate will be 15, 6, and 3 lbs/acre for crimson, red, and ball clovers, respectively. Nitrogen will be applied three weeks after planting (30 lbs N/acre) and after the first grazing event (70 lbs N/acre; 50% as ESN and 50% as urea) on plots fertilized with 100 lbs N/acre rate. Other nutrients will be applied following IFAS recommendation for the specific crop/cover crop.

Nitrate leaching was monitored with drain and porous cup lysimeters. Nitrate sampling followed a similar protocol described for the first experiment. Herbage mass and nutritive value were determined following Dubeux et al. (2016). Cattle performance were determined every 21 days after a 16-h fasting period. Cattle average daily gain (ADG) was calculated for each 21-d period by dividing the average weight gain of the two tester animals during that specific period by the number of days. Grazing days were calculated by multiplying the number of livestock in each paddock by the number of days within each period. Numbers were adjusted to express grazing days per acre. Gain per area were estimated by multiplying the average daily gain by the grazing
days per acre within each period. Stocking rate was estimated by dividing the total cattle BW per paddock by 1000 lbs, which is defined as one animal unit (AU).

**Results and Discussion: Nitrates**

Drain gauges were installed in late June 2019. There was little to any leachate production from June until August 2019. By mid-August, leachate production was becoming more routine. One of the challenges in obtaining a real representation of leachate NO3-N is the high level of within-treatment variability. However, repeated, longer term sampling helps to better illustrate treatment impacts. For example, Figure 37A represents leachate volume, N loading, and leachate NO3-N concentration across treatments. Even with frequent, large error bars, patterns present themselves. Leachate volume tended to track rainfall, particularly beginning in 2020. Prior to 2020, the Low N Bahia treatment did not track leachate volumes as well as the other treatments and appeared excessive during the 2020 winter. In comparison, the Bah+RP treatment trended lower, in terms of leachate volume and yet it also tracked rainfall events well.

Greater leachate volume often results in greater NO3-N loss via leachate. Figure 37B illustrates this well. The Bah + RP NO3-N loss was consistently low compared to the other bahiagrass systems, but greater leaching from the low N Bahia treatment in the 2020 winter also coincided with greater NO3-N loss. Li et al. (2018) concluded that irrigation has greater influence on nutrient leaching risks and thereby, environmental risk than N fertilizer inputs. By winter 2021, the high N treatment also resulted in greater N leachate losses. This was particularly noticeable with the Oct 2020 rain event, where NO3-N loss was calculated as great as 40 kg N ha\(^{-1}\). This value was skewed to one replicate producing excessive quantities of leachate at moderately high (over 20 mg N L\(^{-1}\)) concentrations (Figure 37C). Figure 38 illustrates the large change in ETo from winter, when plant transpiration losses are relatively low, compared to summer months, when ETo often meets or exceeds rainfall quantities. If there is not enough water to allow for leaching, then regardless of soil NO3-N loading, it will tend to remain onsite. This is tempered by existing soil moisture at the time of a rainfall event, rainfall rate, and soil infiltration. Additionally, macropores, due to root channels, earthworms, and other soil disturbances impact water infiltration and movement (Ghodrati et al., 1999; Katsvairo et al., 2007).

Seasonality also impacts the likelihood of greater NO3-N leaching losses. It is observed in Figure 37 that greater N losses tended to occur during the winter months (approximately November through March), regardless of N treatment. Even with significant summer rain events, N loss was often limited. For example, N loss was rarely more than 10 kg ha\(^{-1}\) in mid-summer, while it often exceeded 20 kg N ha\(^{-1}\) during winter months.

Regardless of season, the Bah + RP treatment (bahiagrass with rhizoma peanut) consistently resulted in in low leachate yield with corresponding low NO3-N concentrations (remaining below 20 mg L\(^{-1}\)). There was only one sampling event (Mar 2021) when N loss from Bah + RP approached 20 kg N ha\(^{-1}\). In the low N Bahia and Bahia + RP, low mineral N rates were applied
but the low N Bahia (based upon the drain gauge data) tended to lose more soil N as leachate. Legume inclusion tends to be associated with greater soil aggregation, perhaps through interactions with mycorrhizae and other organisms (Barea and Azcon-Aguilar, 1983) and aggregates help conserve N (Cambardella and Elliot, 1993).

The cost and maintenance of drain gauge lysimeters is high. In comparison, porous cup lysimeters are less costly but have large limitations relative to quantifying N leaching losses, since samples represent soil pore water recovered under a vacuum rather than free draining leachate and the porous cup zone of influence for estimating representative area is difficult to determine and is often unachievable. However, due to their much lower cost and ease of installation and sampling, they can be useful additions for monitoring treatment differences. Adequate lysimeter-based nutrient tracking of NO₃-N losses with rain or irrigation events have been reported, particularly under low to moderate N application rates (Zotarelli et al., 2007). However, with high rainfall events, porous cup lysimeter N recovery is often less than other methods. Interestingly, we found greater NO₃-N concentrations in porous cup lysimeter soil pore water than leachate water collected from the drain gauge at the highest N treatment, Bah + N (Figure 39). However, porous cup lysimeter values were often lower than drain gauge values for the other two treatments. Regardless, the trend of greatest N loss from the Bah + N treatment and least loss from the Bah + RP treatment was consistent with drain gauge data. These data support the continued discretionary use of porous cup lysimeters to help track trends, particularly among different treatments, while drain gauges, used strategically, can provide data to help verify soil transport models that will be needed for addressing NO₃-N leaching sources and impacts at larger-scale (i.e., watershed, regional).
Figure 37. Drain gauge time-course data presented as leachate (A), leached inorganic N (NO$_3$-N + NH$_4$-N) (B), and leachate NO$_3$-N concentration (C) from August 2019 through June 2021. Each symbol represents the mean of 3 replicates ± standard error. Some samplings had single or zero rep if there was no sample to collect. Rainfall data (hatch-line) was retrieved from the Florida Automated Weather Network (FAWN). Bah + N=bahiagrass supplied with 112 kg N ha$^{-1}$ in mineral form and no legumes; Low N bah=bahiagrass + 34 kg N ha$^{-1}$ + cool-season legumes; Bah + RP=bahiagrass + 34 kg N ha$^{-1}$ + rhizoma peanut + cool-season legumes.
Figure 38. Rainfall and reference evapotranspiration (ETo) retrieved from UF-IFAS FAWN weather station located at NFREC-Marianna from 01 August 2019 through 30 June 2021. Each symbol represents the summation between it and the previous symbol (first values used period between 00 and 08 August 2019). Some samplings had single or 1 rep if there was no sample to collect. Rainfall data (a) was retrieved from the Florida Automated Weather Network (FAWN). Blue boxes bracket the estimated winter months (November through March), when ETo is lowest.
Figure 39. Time-course data comparing porous cup with drain gauge derived NO3-N concentrations in soil water for three forage treatments: Bah + N=bahiagrass supplied with 112 kg N ha\(^{-1}\) in mineral form and no legumes; Low N bah=bahiagrass + 34 kg N ha\(^{-1}\) + cool-season legumes; Bah + RP=bahiagrass + 34 kg N ha\(^{-1}\) + rhizoma peanut + cool-season legumes. Each bar represents the mean of 3 replicates ± standard error. Some samplings had single or zero rep if there was no sample to collect.
7. PRODUCTS FROM THE PROJECT

Graduate students

1. Erick Santos, PhD
2. David Jaramillo, PhD
3. Liza Garcia, PhD
4. Victor Guerra, PhD

Peer-reviewed publication published in scientific Journals

   http://doi.org/10.1002/csc2.20568

   https://doi.org/10.1002/agj2.20675


Abstracts presented in scientific meetings


fertilized grass or grass-legume pastures in North Florida. 7th Greenhouse Gas and Animal Agriculture Conference, 4-10 August 2019, Foz do Iguassu, Brazil.

Book Chapters


Extension reports


EDIS publications

117

Educational efforts and activities

- 2021 Forage Legume Webinar Series (5).
- 2020 Video for the Panhandle Ag highlighting the “Integration of Forage Legumes into livestock systems” https://youtu.be/QTgV9pyD7Z4
- 2020. Ecosystem services delivered by forage legumes in grassland ecosystems. 01 June 2020. Available at Servicios AmbientaisProvididos por Leguminosas Forrageira em Ecossistemas de Pastagens - YouTube
- 2020. Management of grass-legume mixtures to optimize the benefits to the soil. 22 September 2020. Available at PALESTRA 3 - IV SIMPRUPASTO 22 DE SETEMBRO DE 2020 - YouTube
- 2020 Video for the cover crop virtual field day. Grazing management of cover crops. Video available at Dr. Jose Dubeux-Grazing Cover Crops - YouTube
- 2020. Grassland ecosystems and their environmental benefits. Available at CONEXÃO UNIV. FLÓRIDA x BRASIL - Tema: Os ecossistemas de Pastagens e seus Benefícios Ambientais - YouTube
- 2020 Beef and Forage IST. Invited speaker addressing stockpiling of limpograss for extension professionals.
- 2020 Heifer Development Field Day. Invited speaker addressing cost of supplementation and forage options to extend the grazing season. 10 Jan. 2020
- 2019 Perennial Peanut Field Day at Quincy, with field tour for producers. We had 95 attendees.
- 2019 Beef and Forage Field Day at UF IFAS NFREC Marianna. We presented successful stories of integrating forage legumes in livestock systems. We had 68 attendees.
- 2019 Escambia County Field Day, addressing cool-season forage legumes, with 30 attendees.
- 2019 joint Webinar Auburn University - University of Georgia - University of Florida on alfalfa-bermudagrass mixtures with 40 attendees.
- 2019 Florida Cattlemen’s Association Year End Quarterly Meeting, invited speaker (FCA
Researcher of the Year in 2019) addressing FCA Board highlighting my research and extension program.

- **2019 Beef and Forage Field Day at UF IFAS NFREC Marianna**, we presented results of our crop-livestock system project. We had 68 attendees.
- **2019 IST Grazing Management of Cover Crops**, coordination of IST and speaker addressing “Grazing cover crops in croplands: opportunities, benefits, and challenges”. 9 attendees (extension faculty).
- **2018 Forage Legume Conference**, a regional conference with the goal to increase forage legume adoption among livestock producers. The program occurred in 15 March 2018 in Marianna, FL and had 105 attendees. Exit surveys demonstrated high levels of satisfaction among attendees, with 75% rating the conference as excellent and 25% as good, 100% learned new things and 82% plan to apply the new knowledge in their operation. We planned to organize this conference every other year, with the next one planned for 2020. Because of COVID-19 we had to postpone it. This became a regional conference on forage legumes with key speakers from SE US States.
- **2018 Forage Workers Tour** (in-service training) with 48 attendees including county faculty and statewide research specialists.
- **2018 North Florida Grazing School**, with 44 attendees including producers, county faculty and statewide specialists.
- **2018 Florida Dairy Production Conference**, invited speaker highlighting the forage options for FL dairy producers (120 attendees).
- Perennial Peanut Producers Association Field Day 2018, 2019, 2020 (co-host at NFREC, Quincy), First week of June. Covered perennial peanut ornamental use, hay production and use in pastures.
- **Agronomic In-service training 2018, 2019, 2020** (co-host at NFREC, Quincy), third week of January. Covered BMPs, cropping systems, and pests and diseases.
- **Beef and Forage In-service training 2020** (co-host at Marianna, FL), third week of January. Covered BMPs, animal management, and pasture management.
- **Soil health In-service training 2021** (co-host virtual event, Quincy, FL), 22 February. Covered BMPs and different aspects related to building soil carbon in row crop land and pastures.
- **2018 Silage and Forage Field Day at Citra**, invited speaker highlighting the use of forage legumes (100 attendees).
- **2018 FFA leadership tour**, class with 18 students highlighting importance of agriculture and livestock systems in Florida.
- **2017 Beef and Forage Field Day**, displaying forage legume trials at UF/IFAS NFREC in Marianna (105 attendees).
- **2017 FDACS Research Committee Seminar**, invited speaker to present updates on the use of perennial peanut in grazing systems. NRCS established cost-share program to strip-plant perennial peanut after revising our data.
- **2017 Forage Workers Tour**, IST with extension professionals at Citra and Ocala (40 attendees).
- **2017 Extension Academy**, coordination with Dr. Brent Sellers of the extension academy, training extension professionals on forages and weeds ID. The IST occurred in three locations: Live Oak, Marianna, and Jay.
• 2017 Perennial Peanut Field Day at Ona, speaker highlighting our SARE on-farm project in collaboration with Dr. J. Vendramini.
• 2017 FFA leadership tour, class with 20 students highlighting importance of agriculture and livestock systems in Florida.
• 2017 Beef and Forage Field Day, displaying forage trials at UF/IFAS NFREC in Marianna (105 attendees).
• 2017 Southern Pastures Conference, invited speaker addressing ecosystem services of grasslands. 60 attendees.
• 2017 Field Day Black Oats and Cool-Season Forages, field day at Shenandoah and UF Dairy for extension professionals and producers. 30 attendees.
• 2017 Cattlemen Tour in Marianna, Leadership Academy Group of the FCA visited our research plots in Marianna.
• 2017 NW Hay Conference, invited speaker addressing Decision Making for Variety Selection.
• 2017 Agronomy IST in Quincy, addressed Forage Management updates.

Mackowiak, C.L. and J. Shirley. 2018. Soil type and grass influences on perennial peanut. 31 May, Perennial Peanut Field Day, Quincy, FL (presentation).
Mackowiak, C.L. and V. Guerra. 2018. Florida soils and their protection. 06 Oct. NFREC Fall Festival, Quincy, FL (demonstrations)
• Mackowiak, C.L. 2020. Update in-field measurements to track forage quality and nitrates 05 Mar, Winter forages field day, Live Oak and Mayo, FL (presentation).

**Success stories**

A major positive impact from this extension program is the increased adoption of forage legumes. We were able to reduce N fertilizer input from 200 lb N/acre to 30 lb N/acre, and to maintain livestock productivity by using grass-legume based systems. This has a major economic and environmental implication for beef cattle production systems in Florida. Assuming a reduction of 170 lb N/acre, 500,000 acres of pastures using N fertilization, and $0.5/lb N, we have a cost savings of $42.5 million dollars annually and a reduction on nitrate leaching on FL groundwater. In addition, we are measuring a myriad of ecosystem services (ES) in contrasting grazing systems with the goal of putting an economic value on these ES. The long-term goal is to find mechanisms to pay land managers for the ES their grasslands provide to society.

We contacted the NRCS State Agronomist to include the establishment of strip-planted rhizoma peanut in bahiagrass pastures in the EQIP program based on our research data. They accepted our request and now this cost-share program is available for producers throughout the State of Florida. The program funds $254 per acre of planted strip of rhizoma peanut.

Our project on integrated crop-livestock systems shows the potential to utilize 700,000 acres of row crops in FL during the cool-season, with an estimated economic value of $480 per acre, resulting in 336 million dollars if fully adopted. In Jackson County, Mr. Steve “Beaver” Yoder is currently leasing row cropland during the fall and using stocker steers for grazing. This is a great example of how the integration of row crop and livestock systems might help the region. In 2019, Mr. Yoder had approximately 2,000 stockers using this system.

Several producers are now integrating perennial peanut into their grazing systems, and some are adopting integrated crop-livestock systems. Below are the testimonials of a few of them, indicating the value of our extension program.
8. REFERENCES


grass mixtures in north florida. Crop Science, 56(5), 2841-2852. doi:10.2135/cropsci2016.03.0141


128


131


