Influence of Plant-Growth Promoting Rhizobacteria on Arthropod Populations, Forage Biomass, and Soil Health in Bermudagrass Pastures

by

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Abstract

Bermudagrass (*Cynodon dactylon* (L.) Pers.) is a warm-season, perennial grass commonly used in forage production systems in the southeastern United States. Drought tolerance and pronounced yield response to high rates of nitrogen fertilizer applications make bermudagrass one of the preferred species for hay production and grazing pastures. With growing concerns of negative environmental impacts resulting from high levels of nitrogen fertilizer applications, it is important to explore biological alternatives for fertilizers in the forage industry. Plant growth-promoting rhizobacteria (PGPR) represent a potential sustainable alternative to chemical fertilizers that could significantly reduce the amount of nitrogen in forage production. The overall purpose of this project was to evaluate the potential of PGPR as a biostimulant to reduce chemical inputs in bermudagrass hay fields and grazing pastures. Plant growth promoting rhizobacteria with and without nitrogen fertilizers were compared to full rates of nitrogen fertilizers to evaluate differences in forage biomass, arthropod populations, and soil health among treatments.

Chapter I is a detailed review of literature pertaining to bermudagrass, major forage pests, nitrogen fertilizers, and PGPR. This review provides background on bermudagrass, its improved varieties, and management practices utilized in hay production and grazing systems in Alabama. An overview of forage pest management, including sections discussing history and biology of the fall armyworm (*Spodoptera frugiperda*) and the bermudagrass stem maggot (*Atherigona reversura*), are also included. A section discussing negative environmental impacts of nitrogen fertilizers precede a section discussing PGPR, how they work, and their potential for reducing chemical inputs in the agricultural industry.

In Chapter II, a 2-year, large-scale field evaluation in bermudagrass fields comparing effects of PGPR and nitrogen fertilizers was conducted. Three grazed locations and three hay locations throughout the state of Alabama were established with three replications per location and six treatments per replication. The six treatments include a blend of PGPR (Blend 20), a single strain PGPR (DH44), Blend 20 plus a ½ rate of nitrogen fertilizer, DH44 plus a ½ rate of nitrogen fertilizer, a full rate of nitrogen fertilizer, and a control plot. These treatments allowed comparisons of PGPR to a full rate of nitrogen, and an evaluation of the effect of PGPR when applied with a half rate of nitrogen fertilizer. Full and half rates of N for bermudagrass were based on extension recommendations in Alabama. Two applications of treatments and four harvests were completed each year. At harvests, arthropod populations, forage heights, and forage dry weight data were collected. Treatments significantly affected biomass weights at location managed for hay production and grazing. Forage weight from plots treated with the full rate of nitrogen fertilizer and both PGPR plus ½ rate treatments was significantly greater than control plots. These results indicate that PGPR plus a ½ of nitrogen fertilizer can yield similar results as a full rate of nitrogen fertilizer in fields utilized for hay production and grazing systems.

In Chapter III, a 1-year, large-scale field evaluation in bermudagrass pastures comparing effects of PGPR and nitrogen fertilizers was conducted. Two sites at the E.V. Smith Research Center (Macon County, Alabama) were selected and three replicates of six treatments were established on each site. The treatments were Blend 20, DH44, Blend 20 plus a ½ rate of nitrogen fertilizer, DH44 plus a ½ rate of nitrogen fertilizer, a full rate of nitrogen fertilizer, and an untreated control. These treatments allowed comparisons of PGPR to a full rate of nitrogen, and an evaluation of the effect of PGPR when applied with a half rate of nitrogen fertilizer. Two applications of each treatment were applied to plots and were evaluated four times. At harvests, arthropod populations, forage heights, and forage dry weight data were collected. In September 2020, soil cores and samples were also collected from each plot to analyze and compare populations of the soil mesofauna and soil respiration as indicators of soil health. There were no significant treatment differences for forage biomass or arthropod populations at either site. Soil respirations and populations of mesofauna were numerically greater in plots treated with PGPR but were not statistically significant in oribatid populations.

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I would also like to thank those that had a strong impact on me during my bachelor's degree. My undergraduate journey was not an easy one. If not for my family and a few very special individuals from Auburn University, I would have never considered pursuing a master's degree. I'll start by thanking my parents. Without their love and support, college would have never been an obtainable goal for me. I am extremely blessed to have wonderful parents that not only did an awesome job of raising my siblings and myself, but also ensured we had the

opportunity to pursue our dreams. Through many ups and downs, they have continued to be our number one fans! They have gone above and beyond to help my sisters and I through our college careers and I could never thank them enough. I would also like to thank my sisters, Mary Sullins-Fuller and Lacey Sullins-Smith, and my brother and sister-in-law, Nick Sullins and Madalene Hayes-Sullins for their love and support throughout graduate school. Thank you all for always believing in me and supporting me no matter what!

In the agronomy department, classes taught by Dr. Eve Brantley and Dr. Matthew Waters made me realize I had an intense interest in factors that negatively impact our environment. In addition to their awesome class content, Dr. Brantley and Dr. Waters also helped me through a rather tragic personal event that occurred while I was enrolled in their classes. Their compassion and understanding reignited my motivation to not only finish college, but to pursue a career in something I am passionate about. After their classes, I had a new perspective for my future career goals and quickly realized I wanted to pursue a master’s degree.

The following year, I worked a summer job with Mr. Jim Harris at the AU turf unit. I was not originally interested in working with turfgrass, but Mr. Jim made me realize that turfgrass is a fascinating industry with many working parts. We primarily focused on weed control, however, the fertilizer studies were what interested me most. I had taken a class by Dr. Beth Guertal that had originally sparked my interest in nutrient management. The knowledge obtained in her class along with the field studies with Mr. Jim lead to my increased interest in fertilizers and their effects. Mr. Jim knew my passions did not lie within weed science, but he supported me through the entire process of finding a master’s project that aligned with my interests and has been one of my number one fans since the day we met. I will always be thankful for the experience I gained while working for him that summer and for his kindness and support throughout the years.

After working for Mr. Jim, I was enrolled in Dr. Held's economic entomology class for the fall semester. I was not super excited about this class because I had never had an interest in insects at all. However, his lectures on biological controls and the negative impacts of the overuse and misuse of chemical pesticides fascinated me. Throughout that semester, he somehow managed to spark an interest in entomological studies in a girl who is deathly terrified of cockroaches. I started discussing the potential of master's studies with him and that is when I first learned of PGPR. The next semester, I conducted an undergraduate research project in his lab where we studied PGPR colonization in bermudagrass in a greenhouse setting. I quickly realized that PGPR was my biggest passion to date. It tied in the interests of environmental impacts, turfgrass, and entomology all into one fascinating project. After that, I began the process of starting this master's degree and the rest is history.

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Dr. Leanne Dillard for her patience and understanding with me throughout this project. Being an entomology major, I have had many questions about the forage aspect of this project. Dr. Dillard has never hesitated to answer my questions, no matter how dumb they may sound. She also supported me through my mid-project mental breakdown and has always exhibited confidence in my ability to complete this project. For that I am extremely grateful.

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cfu	Colonizing Forming Units
CP	Crude Protein
DM	Dry Matter
PGPR	Plant-Growth Promoting Rhizobacteria
BSM	Bermudagrass Stem Maggot
FAW	Fall Armyworm
IPM	Integrated Pest Managed
WG	Wiregrass
CC	Clay County
LC	Lawrence County
MC	Macon County
EVS	E.V. Smith Research Center

Chapter I:
Review of Literature

Bermudagrass - History and Background:

In the southeastern United States, warm-season grasses are the primary forage source used in livestock production systems (Pitman and Alison 2020). Bermudagrass (*Cynodon dactylon* (L.) Pers.) is a warm-season, perennial grass commonly utilized for hay production and grazing in the southeastern United States due to its pronounced yield response to nitrogen fertilizer applications (Lemus 2018). Bermudagrass also creates an extensive root system increasing drought tolerance (Redfearn and Rice 2014). Improved varieties of bermudagrass have enabled greater forage yield, nutritional value, and flexibility within the forage system (Pitman and Alison 2020). Two promising varieties that are increasingly used for hay production and grazing pastures are `Tifton 85’ bermudagrass and `Russell’ bermudagrass.

Tifton 85 bermudagrass was released as an improved hybrid bermudagrass in the United States in 1992. The creation of the Tifton 85 hybrid began with evaluations of bermudagrass strains collected from Africa in the 1960s (Burton 2001). Based on weight gain of fall armyworm and resistance to damage from the insect, `Tifton 68’ from Kenya and `Tifton 292’ from South Africa were chosen to be cross bred and evaluated. Out of 145 F1 hybrids created, Tifton 85 was selected and named based on dry weight accumulation and drought tolerance (Burton 2001). When compared to other bermudagrass hybrids, Tifton 85 yields greater forage dry matter (DM) accumulation as well as nutritional value (Liu et al. 2011). In fact, according to the USDA website, Tifton 85 is the top rated F1 hybrid bermudagrass currently available in the United States for forage production (Burton and Utley 2016) and acreage of Tifton 85 for hay production and grazing continues to increase (Liu et al. 2011).

Another promising bermudagrass variety available is Russell bermudagrass. Russell was first identified in 1977 on a farm in Russell County, Alabama in a `Callie' bermudagrass field and is believed to be the result of natural hybridization (Ball et al. 1996). Compared to other bermudagrass varieties currently available in Alabama, Russell spreads just as fast or faster than most varieties except Tifton 85. Russell also exhibits earlier spring green up and better winter hardiness than most other varieties (Ball et al. 1996). This makes it an excellent selection for hay production and grazing pastures in Alabama.

Major Pests of Bermudagrass:

Pest management is important to reduce forage biomass loss, particularly to insect pests. In forage production systems, a wide variety of insect species can negatively impact the grasses due to the favorable conditions and ample food source provided by the forage (Sulc et al. 2020). Arthropod pests can cause damage to the bermudagrass by damaging roots during burrowing, sucking liquids from the plant, or by feeding directly on the leaves and stems (Vittum 2020). Insect "damage" occurs when activity of the insect populations causes plant injury resulting in economic loss (Sulc et al. 2020). In Alabama, integrated pest management (IPM) strategies for forage production systems are primarily focus on 11 species. Those species include fall armyworms (Lepidoptera: Noctuidae), green June beetles and other white grubs (Coleoptera: Scarabaeidae), sugarcane beetles (Coleoptera: Scarabaeidae), billbugs and clover head weevils (Coleoptera: Curculionidae), two-lined spittlebugs (Hemiptera: Cercopidae), chinch bugs (Hemiptera: Blissidae), fire ants (Hymenoptera: Formicidae), winter grain mites (Trombidiformes: Penthaleidae), bermudagrass stem maggots (Diptera: Muscidae), and striped ground crickets (Orthoptera: Trigonidiidae) (Kesheimer 2021).

Of these pests, the fall armyworm (FAW) and the bermudagrass stem maggot (BSM) are the most economically important because they can cause large economic losses each year (Lemus 2017). Both insects have immature forms that can cause significant damage to the top growth of bermudagrass.

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is an arthropod pest that is native to the Americas (Vittum 2020). These arthropods are generalist herbivores with over 60 identified host plants and the larvae have been known to cause significant damage to bermudagrass in the southern United States (Hong et al. 2015). Fall armyworms cannot survive the winter months in most areas of the continental United States, so they overwinter in southern parts of Florida and Texas. When temperatures increase in spring, the FAWs are redistributed over a majority of the eastern United States and parts of southern Canada (Johnson 1987). First occurrences of FAW damage are typically reported in late July or early August in southern Alabama (Vittum 2020). The fall armyworm typically has three or more generations per year in Alabama, which can result in significant economic losses for producers in hay production and cattle grazing (Meagher et al. 2007).

From egg hatch until death, the FAW typically completes its life cycle in approximately 30 days during summer months, 14 of which are in the larval development phase (Hong et al. 2015). The adult fall armyworm is an ash-gray colored moth with front wings mottled with gray and white spots. After egg hatch, immatures appear as tiny, light-colored larvae with a black head. However, their body will darken as they mature, and they will develop a signature inverted “Y” on their head (Vittum 2020). Fall armyworms have exceptionally high rates of reproduction with females depositing around 900-1,000 eggs each (Johnson 1987). The adult females lay eggs at night in masses on lightly colored structures, such as buildings or fence posts, or on the bermudagrass leaves and sheaths (Vittum 2020). The oviposition for females can last up to 17 days, with a majority of eggs being deposited within the first 4 to 5 days. After oviposition, eggs typically hatch after 2 days of development, but can take up to 17 days. Development time of immature stages is dependent on temperature but is usually faster than many grass feeding caterpillars (Johnson 1987). After egg hatch, the larvae feed for 14 days, with the most significant feeding period during the 6th instar, or the last 2-4 days of the period. The immature fall armyworms damage the bermudagrass by feeding directly on the green and tender areas of the forage, leaving tough stems and jagged leaf edges behind (Vittum 2020). Fall armyworms can cause devastating damage to bermudagrass hay fields and pastures within a few days if not identified and controlled early. Sweep samples from areas where damage is observed is an effective tool for identifying the presence of FAWs in a field (Kesheimer 2021). The best pest management strategy is the application of insecticides at the first site of damage. Recommended insecticides vary depending on life phase, but pyrethroids are the most commonly used (Vittum 2020).

Another significant pest of bermudagrass forage in the southeastern United States is the bermudagrass stem maggot (BSM). The bermudagrass stem maggot, *Atherigona reversura* Villenueve (Diptera: Muscidae), is an invasive species first discovered in Georgia in 2010 (Baxter et al. 2014). They are grass specialists with only two identified host plants, bermudagrass (*Cynodon dactylon* (L.) Pers.) and stargrass (*Heteranthera zosterifolia*) (Hudson et al. 2019). The introduction of BSM in Georgia in 2010 has caused significant reductions in accumulated forage and nutritional values between the months of July and September (Baxter et al. 2019). Since then, its range has expanded to most states in the southeastern United States (Baxter et al. 2014). The BSM maggots are small and hard to sample and identify. The adults are small flies with transparent wings, a dark head, and yellow abdomen with a pair of small black spots (Baxter et al. 2014). The adult stage is easier to collect and identify in samples.

The life cycle of a bermudagrass stem maggot is typically around 3 weeks (Hudson et al. 2019). The adult females lay eggs on the stem of bermudagrass near a node. After egg hatch, the maggot will burrow into the shoot of the grass and begin feeding until they are ready to pupate, at which point they will drop into the soil to complete development. After the maggots vacate, the shoot and the top two or three leaves directly above the shoot wither and die (Hudson et al. 2019). In large populations, this creates a bronzing or frosted effect on bermudagrass fields and pastures due to the high amounts of dead shoots and top leaves. In severe cases, estimated yield losses of 20 to 50% have been reported (Lemus 2017). By the time producers observe damage, the tiny BSM larvae has already dropped into the soil to pupate, making sampling and pest identification difficult. The bronzing effect from BSM is often mistaken as other stressors, such as plant pathogens, nutrient deficiencies, or drought stress (Baxter et al. 2014). However, the damaged shoots will have a hole at the node below the damage where the maggot exited the shoot, indicating BSM caused the damage. (Hudson et al. 2019). An economic threshold for BSM has not yet been identified (Kesheimer 2021). In fields with high populations of BSM, it is recommended to apply low rates of pyrethroid insecticides labeled for fall armyworm control 5 to 7 days after harvest, with second applications if necessary (Lemus 2017).

Management of Bermudagrass:

In addition to pest management, it is also important to acknowledge the different management strategies used for hay production and pastures managed for grazing. In fields with existing bermudagrass stands, the start of grazing and hay management is based on spring green-up of the bermudagrass. In Alabama, this typically occurs in March (Lemus 2018).

Cattle grazing typically begins when the bermudagrass stand reaches between 10 to 13 cm (around 4 to 5 inches) (Redfearn and Rice 2014). Thereafter, pastures can be stocked continuously or rotationally. Continuously stocked systems consist of one pasture grazed continuously by cattle throughout the season, whereas a rotationally stocked system moves cattle between two or more pastures. There are pros and cons to each of these management methods, but stocking rate remains vital to both (Sollenberger et al. 2020a). With a proper stocking rate, continuously stocked pastures typically result in better animal performance. Continuously stocked pastures also require less management and inputs, therefore that is the most commonly used grazing management practice (Redfearn and Rice 2014). However, in terms of bermudagrass health, rotationally stocked pastures yield better results (Lemus 2018). This is because in a continuously grazed pasture, there is no control of when and where the cattle are grazing. This challenges optimizing forage utilization and often results in uneven growth rates of the bermudagrass (Barnhart et al. 2013). For this reason, rotationally stocked pastures with 10-day rotation intervals are typically recommended (Lemus 2018). This allows the bermudagrass to recover from the grazing activities and optimizes forage utilization (Redfearn and Rice 2014).

In hay production systems, bermudagrass is well adapted, exhibits rapid growth, and responds well to fertilizer applications (Sollenberger et al. 2020b). After greening-up, the bermudagrass should be harvested once the hay has reached a height of 20-25 cm (8-10 in) (Redfearn and Rice 2014). After the first harvest, hay should be harvested every 4 weeks thereafter (Lemus 2018). At harvest intervals of 4-5 weeks, crude protein (CP) and digestibility of the hay are optimized (Redfearn and Rice 2014). The CP content and digestibility of hay is primarily dependent on plant maturity and nitrogen fertilization (Lemus 2018). Therefore, it is crucial to maintain a consistent fertilization schedule in addition to harvest intervals.

Previous experiments indicate that yield and quality of bermudagrass typically respond positively to high rates of nitrogen fertilizer (Kering et al. 2011). In hay production systems, when 112 kg N/ha (100 lb N/acre) is applied and bermudagrass is harvested at 4 weeks, CP of the hay is optimized (Redfearn and Rice 2014). However, the recommended rate for hay production systems is not the same for grazed pasture management. In hay systems, nutrients are removed from the system through hay harvesting (Brink et al. 2003). However, in grazed systems, the nutrients are being recycled through the animal and reapplied to the system in the form of manure and urine (Sollenberger et al. 2020b). The recommended nitrogen rate in grazed systems is 84 kg N/ha (75 lb N/acre) as needed in Alabama (Dillard et al. 2020). In addition to nitrogen requirements, maintaining adequate soil potassium is crucial in bermudagrass systems as well (Barker and Culman 2020). It is recommended to apply potash (CK_2O) at a rate of 35 to 45 kg/ha/ton of hay harvested (Lemus 2018). While chemical fertilizer applications are necessary to optimize forage utilization, it is also important to consider the environmental impacts of these agrochemicals.

Environmental Impacts of Nitrogen Fertilizer:

Since the 1960s, there has been significant public concern regarding food and fiber production involving chemical fertilizers and pesticides (Nelson et al. 2020). While there have been many laws and industry guidelines set forth to limit the chemical applications utilized in the ag industry, these typically focus on direct health concerns and economic impacts rather than long-term environmental effects (Sanderson and Liebig 2020). However, the public interest in environmental effects has increased significantly since the late 20th century due to significant losses in ecosystem services (Costanza et al. 2017). While it is important for producers to achieve economic stability through more intense management strategies, it is equally important to consider the tradeoff of environmental stability that often occurs (Sanderson and Liebig 2020). Nitrogen is essential to agricultural production systems, however plants are inefficient at taking up the nitrogen applied. Only a fraction of nitrogen applied (as low as 20%) is actually utilized by plants (Dybas 2005). Excess nutrient applications have led to issues such as soil salinity, heavy metal accumulation, water eutrophication, and accumulation of nitrates (Savci 2012). The environmental effects of these excess nutrients should be factors considered for the future of agriculture and our ecosystems.

One important factor to consider when evaluating effects of chemical fertilizer applications is soil health. Soil health can be defined as “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans, and connects agricultural and soil science to policy, stakeholder needs and sustainable supply-chain management” (Lehmann et al. 2020). Maintaining a healthy soil biota is vital for the plants and animals in the surrounding ecosystems (Nelson et al. 2020). In addition to plants, the soil biota is made up of microorganisms (soil microbes) and animals (soil fauna) that spend all or part of their lives in the soil (Fortuna 2012). Soil fauna are divided into three categories based on their size (Table 1.1); microfauna (<0.1mm), mesofauna (0.1-2mm), and macrofauna (>2mm) (Neher and Barbercheck 2019). The soil biota provides essential ecosystem services that maintain the health of the soil. These services include decomposition of organic matter, nutrient cycling, and pest suppression with soil microbes and mesofauna being key players in these roles (Neher and Barbercheck 2019). Soil microbes and mesofauna are sensitive to changes in the soil, so management and land-use can significantly impact these populations and the ecosystem services they provide (Yao et al. 2006). For example, in some forage systems, intense grazing can result in severe soil degradation (Fterich et al. 2012). A recent study also showed that the application of nitrogen fertilizers can cause cytotoxic areas in the soil resulting in microbial dead zones (Ruiz et al. 2020). Significant population declines in soil mesofauna populations have been observed after applications of nitrogen fertilizers as well (Lohm et al. 1977). In terrestrial ecosystems, soil is one of the less studied resources. In particular, there is a lack of research regarding soil mesofauna, or microarthropods, with an estimate of less than 10% of soil species having been described (Ojeda and Gasca-Pineda 2019). Despite the lack of research, soil mesofauna and microbes are vital components of soil health and populations must be preserved to maintain the health of the surrounding ecosystems (Neher and Barbercheck 2019). As loss of ecosystems services become more apparent, the need to incorporate environmental based rational into chemical applications is more important than ever (Costanza et al. 2017).

Another area of concern regarding chemical fertilizer applications is excess nutrient runoff that settles into water systems. Nitrogen runoff from grasslands and the agricultural industry have caused major issues in coastal regions in the United States for years (Wherley et al. 2015). In grazed pastures, phosphorus runoff is a known issue that causes water eutrophication in both coastal and freshwater systems (Anderson et al. 2020). The excess nutrients in runoff end up in the sediments at the bottom of rivers and streams and are eventually dumped into the ocean. Every spring and summer, massive algal blooms occur in coastal areas because of this nutrient runoff (Dybas 2005). These algal blooms cause dissolved oxygen levels to drop below 2 mg O_2 per liter in some areas, creating hypoxic zones, or dead zones, in which most marine life cannot survive (Dodds 2006). These coastal dead zones are considered one of the most devastating anthropogenic threats to marine life (Altieri and Geban 2015). The largest dead zone in the United States exists in the Gulf of Mexico, where the Mississippi River meets the Gulf (Dodds 2006). This dead zone extends from the mouth of the Mississippi River, across the entire coast of Louisiana, and over into the coast of Texas (Dybas 2005). The National Oceanic and Atmospheric Association began tracking the size of this dead zone annually in 1985. In recent years, this dead zone has exceeded the size of the state of New Jersey, with the largest record being 8,776 square miles in 2017 (NOAA 2021). While the Mississippi dead zone remains the largest in the United States, it is by no means the only area affected by hypoxic zones resulting from excess nutrient runoff (Dybas 2005). In recent years, the Mississippi dead zone was reclassified as the second largest dead zone in the world, with the discovery of an even larger dead zone existing in the Arabian Gulf (Lachkar et al. 2020). Smaller dead zones occurring in coastal areas are also being detected at an alarming rate. In 2005, there were 146 dead zones reported worldwide (Dybas 2005). Each decade, the documented occurrences of dead zones in coastal areas is nearly doubling while severity of existing dead zones continues to increase (Altieri and Gedan 2015). Some coastal areas in Florida have already began implementing restrictions for fertilizer applications near the coast (Kirkpatrick et al. 2014). However, these issues demand a response from the entire population and not only the residence of the areas with significant impacts. Exploring biological alternatives to chemical fertilizers is essential to preventing further deterioration and to begin repairing aquatic health in our coastal areas.

Plant Growth Promoting Rhizobacteria:

With the environmental concerns surrounding chemical fertilizers, an increased interest has been placed on identifying microorganisms with the potential to enhance plant nutrition and soil fertility (Adesemoye et al. 2009). Amongst the search, soil bacteria from the rhizosphere of numerous plants have been isolated and re-applied to a variety of crops, resulting in improved plant growth and development (Baber et al. 2018). These beneficial plant-microbiome interactions have the potential to improve agricultural management practices by potentially providing a sustainable alternative to chemical fertilizer applications (Singh et al. 2019). These microorganisms are referred to as plant growth promoting rhizobacteria (PGPR). The term PGPR was originally termed in the 1980's primarily to describe *Pseudomonas* species utilized for biological control of plant pathogens. Since then, the term PGPR has expanded in meaning and is now used to describe any rhizosphere dwelling bacteria that increases plant growth through one or more mechanisms (Prasad et al. 2019).

Bacteria can have different subtypes based on genetic variance within a species. These subtypes are referred to as strains. Different strains within a species can have variable effects on plant hosts, therefore, PGPR are chosen based on their strain rather than their species (Shukla 2019). The selection of PGPR strains begins with the isolation of hundreds of root-colonizing bacteria obtained from the roots of plants harvested in field conditions. These strains are then screened in a lab for their ability to fix or solubilize nutrients or inhibit phytopathogens *in vitro*. Pure cultures of the promising strains are then applied and evaluated in greenhouse trials to determine which strains will be applied and evaluated at a field trial level (Bhattacharyya and Jha 2012). Genetic analyses are also conducted to identify the species of bacteria. Some common genera of PGPR include *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, and Serratia* (Prasad et al. 2019).

To properly utilize PGPR, a basic understand of bacterial colonization is key (Benizri et al. 2001). In order to express their beneficial effects, PGPR must be able to successfully travel to the rhizosphere, inoculate the roots or root zone, and colonize the plant or root zone at their target site without other factors interrupting the process (Ahemad and Kibret 2014). The rhizosphere of a plant is the area directly surrounding the root zone. This area has a variety of inhabitants including microorganisms such as bacteria, fungi, protozoa, and algae. The most abundant of these microbes are the plant growth promoting rhizobacteria (Prasad et al. 2019). PGPR are often categorized as rhizospheric or endophytic based on the area in which colonization occur. Rhizospheric bacteria colonize the rhizosphere and rhizoplane (root surface), but do not enter the plant tissues. Endophytic bacteria can colonize the apoplastic intracellular spaces within the plant tissues, or endorhiza, in addition to the rhizosphere and rhizoplane (Bhattacharyya and Jha 2012). Furthermore, some endophytic PGPR can travel through the plant vascular system and colonize within plant tissues other than the endorhiza. In 2019, a study conducted on bermudagrass determined two strains of *Bacillus pumilus* and one strain of *Bacillus sphaericus* were capable of not only colonizing the rhizosphere, rhizoplane, and endorhiza, but also the phyllosphere of the grass (Coy et al. 2019a). This ability of some endophytic PGPR to colonize within plant tissue could be advantageous because the PGPR are in constant contact with plant cells, and they are protected from biotic and abiotic factors in the rhizosphere that could otherwise inhibit their beneficial effects (Coy et al. 2019a).

Biotic factors that could negatively impact colonization include predation of PGPR by protozoa and competition from other rhizosphere dwelling microbes (Benizri et al. 2001). Soil bacteria can exist in the rhizosphere in large numbers if there is a carbon source readily available. In soils with high microbial populations but limited carbon sources, the PGPR dwelling in the rhizosphere will have to compete for nutrients (Coy et al. 2019 a). Negative impacts can also occur as a result of abiotic factors, primarily soil type and soil temperature. Plant growth promoting rhizobacteria strains are often adapted to climate factors and soil types from the area in which they were isolated (Shukla 2019). It is important to consider these factors when choosing a strain to apply in a specific location.

After successful colonization, PGPR can enhance plant growth through direct and indirect mechanisms. Direct mechanisms include biological nitrogen fixation, phosphorous solubilization, and production of siderophores and phytohormones. Indirect mechanisms play a role in path of synthesis and include production of antibiotics, cell wall degrading enzymes, and induced systemic resistance (Singh et al. 2019). Most PGPR can be divided into three main groups, or categories, based on their responses to biotic and abiotic factors (Prasad et al. 2019). Plant growth promoting rhizobacteria that enhance nutrient availability and uptake in response to abiotic stresses, such as drought stress or temperature, are categorized as biofertilizers. In this category, PGPR primarily utilized biological nitrogen fixation, solubilization of phosphorous and/or solubilization of potassium as their mode(s) of action (Vessey 2003). Plant growth promoting rhizobacteria that enhance stress tolerance in response to biotic factors, such as plant pathogens or insect pests, are categorized as biopesticides and phytostimulators. In these categories, PGPR typically utilize phytohormones, siderophore production, biocontrol, and/or induced systemic resistance as their mode(s) of action (Prasad et al. 2019). These groups are strictly based on the pgpr respinse to biotic and abiotic factors. Modes of action can vary amongst PGPR strains and are not restricted to the category in which they are listed. Some PGPR strains may also respond to multiple biotic or abiotic factors, in which case they will belong to more than one category (Bhattacharyya and Jha 2012).

Research on PGPR has shown promising results regarding their ability to function as phytostimulators and biopesticides (Shukla 2019). Several strains of *Bacillus, Rhizobium,* and *Paenibacillus*, have exhibited the ability to limit or prevent damage caused by phytopathogens in multiple plant species (Singh et al. 2019). Some PGPR strains, such as *Stenotrophomonas rhizophila* and several *Bacillus* spp., have even proven to reduce populations of plant parasitic nematodes in turfgrass (Groover et al. 2020). Blends of PGPR, such as one referred to as Blend 20, have exhibited significant biopesticidal traits. Blend 20 is comprised of two strains of *Bacillus pumilus* and one strain of *Bacillus sphaericus* and has proven effective at increasing tolerance of bermudagrass to infestations of white grubs (Coleoptera: Scarabaeidae) and tawny mole crickets (Orthoptera: Gryllotalpidae) (Coy et al. 2019b, Coy et al. 2020). In another study where this blend was applied to bermudagrass, the amount of FAW eggs deposited onto the bermudagrass was reduced by 25% of that of the control (Coy et al. 2017). These results indicate that PGPR could be an effective tool to reduce insecticide applications in the forage and turfgrass industries.

In addition to insecticide reduction, PGPR also exhibits the potential to significantly reduce chemical fertilizer applications through utilization as a biofertilizers (Adesemoye et al. 2009). Past research indicates that a majority of successful PGPR isolates increase plant height, root length, and DM production in a number of plants through means of biofertilization (Bhattacharyya and Jha 2012). Of these benefits, the effect on root development and morphology seems to be the most significant (Vessey 2003). Several strains of *Pseudomonas* and *Bacillus* have been successful at increasing drought stress tolerance in agricultural plant species, such as maize (*Zea mays*), tomatoes (*Solanum lycopersicum*), and rice (*Oryza sativa*). These PGPR induced positive changes in the root morphology and architecture that lead to this increased tolerance (Barnawal et al. 2019) In hybrid bermudagrasses, inoculation with blends of *Bacillus* spp. have resulted in significant increases in both root and shoot biomass (Coy et al. 2014). A field study on stockpiled bermudagrass indicated that when applied with half rates of nitrogen fertilizer, treatments including a blend of *Bacillus* spp. and a single strain application of *Paenibacillus* spp. can yield similar forage biomass and nutritional value as a full rate of nitrogen fertilizer (Griffin et al. 2020). Similar results were seen when two single strain application of *Bacillus* spp. were applied to tomatoes plants with a half rate of nitrogen fertilizer as well (Adesemoye et al. 2009). These results indicate promising potential for PGPR utilization to decrease nitrogen rates not only in forage production, but the entire agricultural industry.

In PGPR literature, there are currently concerns with contradicting definitions of the term "biofertilizer" (Mącik et al. 2020). Another term used to describe PGPR that enhance nutrient availability and uptake in response to abiotic stresses is "biostimulant" (Calvo et al. 2014). In most of the literature, PGPR are divided into the three categories listed above. However, the description of PGPR as a “biofertilizer” has been brought into question in recent years (Mącik et al. 2020). Until recently, the definition and concept of a biostimulant was still evolving (Calvo et al. 2014). However, a majority of publications now define biostimulants as “any substance or microorganisms applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content.” (du Jardin 2015.) According to this definition, PGPR described as biofertilizers are indeed biostimulants. The issue then lies in the definition of biofertilizers. This term is defined in various ways throughout literature, and the concept of biofertilizers has changed as the knowledge of soil microbial interactions with plants has progressed (Mącik et al. 2020). Contradicting definitions make it difficult to determine whether PGPR should be referred to as biofertilizers, biostimulants, or both. However, since the definition of a biostimulant seems to be more consistent throughout literature, PGPR that enhance nutrient availability and uptake in response to abiotic stresses will be referred to as “biostimulants” for the remainder of this paper.

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Soil Fauna		
Type	Size	Examples
Microfauna	<0.1 mm	Nematodes and Protozoa
Mesofauna	0.1-2 mm	Mites, Collembola, and Protura
Macrofauna	>2 mm	Earthworms, larger adult and immature insects

Table 1.1 Soil Fauna Categories and Examples

Chapter II:

Effects of Plant-Growth Promoting Rhizobacteria and Nitrogen Fertilizer on Forage Biomass and Arthropod Populations in Bermudagrass Fields in Alabama

Introduction:

Forage production is the second largest industry in Alabama in terms of land-use (Ball 2008). Of the warm-season grasses utilized in hay production and grazing, bermudagrass (*Cynodon dactylon* (L.) Pers) is the most commonly used due to its drought tolerance and pronounced response to nitrogen fertilization (Lemus 2018). Two improved varieties are Tifton 85 and Russell bermudagrass, both of which have increased resistance to stress caused by pests and drought (Liu et al. 2011, Ball et al. 1996). Many arthropod species can cause negative impacts on bermudagrass, but two of the most significant pests in the Southeastern United States are the fall armyworm (FAW), *Spodoptera frugiperda,* and the bermudagrass stem maggot (BSM), *Atherigona reversura*. Both pests have immature forms that can cause significant damage in bermudagrass fields and pastures (Vittum 2020, Kesheimer 2021). Proper bermudagrass management practices can minimize these effects while optimizing forage utilization. Management of bermudagrass for hay production and grazing both begin after the green-up period, which occurs around March in Alabama. Rotational stocking is the recommended management for grazing pastures and hay should be harvested every 4-5 weeks (Lemus 2018). Both management practices, along with appropriately timed nitrogen fertilizer applications optimize forage utilization and crude protein (CP) levels (Sollenberger et al. 2020a).

While nitrogen fertilizers are a vital part of plant production in the agricultural industry, it is also important to consider the negative impacts of these chemicals (Sanderson and Liebig 2020). Depletion of soil health and creation of dead zones in coastal areas are two of the most significant anthropogenic effects resulting from chemical fertilizer applications (Yao et al. 2006, Altieri and Geban 2015). Exploring biological alternatives that result in sustainable agriculture that is ecological sound is essential (Sanderson and Liebig 2020). Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria that promote plant growth through several mechanisms (Prasad et al. 2019). Use of PGPR as a biostimulant has displayed promising potential for reducing chemical fertilizer applications in bermudagrass and the agricultural industry as a whole (Adesemoye et al. 2009).

Previous studies conducted at Auburn University have identified several strains of PGPR that are effective at growth promotion in hybrid bermudagrasses. Inoculation with blends of *Bacillus* spp. have resulted in significant increases in both root and shoot biomass of bermudagrass in greenhouse trials (Coy et al. 2014). A field study on stockpiled bermudagrass indicated that when applied with half rates of nitrogen fertilizer, treatments including a blend of *Bacillus* spp. and a single strain application of *Paenibacillus* spp. can yield similar results as a full rate of nitrogen fertilizer about forage biomass and nutritional value (Griffin et al. 2020). Considering these results, the goal of this study was to evaluate the potential of PGPR to reduce chemical fertilizer rates needed in forage production by comparing PGPR treatments to treatments of nitrogen fertilizer. In particular, the objectives of this study were to (1) compare the effects of PGPR and nitrogen fertilizers on forage biomass in bermudagrass fields utilized for hay production and grazing and (2) compare the effects of PGPR and nitrogen fertilizers on arthropod populations in bermudagrass hay fields.

Materials and Methods:

PGPR Strains:

For this study, a blend of three *Bacillus* spp., known as Blend 20, and a single strain application of *Paenibacillus* sp., were applied as the PGPR treatments. Blend 20 consists of two strains of *Bacillus pumilus* (AP7 and AP18), and a strain of *Bacillus sphaericus* (AP282). The single strain application of *Paenibacillus* sp. DH44, a species closely related to *Paenibacillus sonchi*. These bacteria were grown in the lab and scraped into solution for field applications. All strains were grown in an incubator at 28°C for their optimal growth dates. AP282, *Bacillus sphaericus*, was allowed to grow for three days before scraping. AP7 and AP18, *Bacillus pumilus*, were grown for 5 days. And DH44, *Paenibacillus* spp., was grown for 10-12 days before scraping. Upon the optimal growth date, the bacteria were scraped into solution using autoclaved de-ionized water. After being scraped into 250mL bottles, the PGPR were soaked in a water bath at 80°C to kill off the vegetative phase and leave the endospores for field applications. Blend 20 was applied at a concentration of 1×10^7 and DH44 was applied at a concentration of 5×10^6.

Field Evaluation:

Field trials for this study were conducted from May through October of 2020 and 2021 at six bermudagrass fields throughout Alabama to evaluate effects of PGPR and nitrogen fertilizers. Three locations were bermudagrass fields utilized for hay production and three were grazed pastures. The hay sites were located in Dale County, Clay County, and Lawrence County. While the grazed sites were in Houston County, Clay County, and Macon County. Grazed sites had cattle active in the same pasture, but cattle were excluded from this study site by electrified fencing. Figure 2.1 shows a map of Alabama with the counties included in this study highlighted. Table 2.1

shows locations and soil types as indicate by the NRSA web soil survey as well as cultivar and management practices for each site.

The experimental design was an augmented factorial randomized block design with six treatments in 1 m^2 plots and three replicates per location. The six treatments included blend 20 (B20), B20 plus a half rate of nitrogen fertilizer (B20 + ½ N), DH44, DH44 plus a half rate of nitrogen fertilizer (DH44 + ½ N), a full rate of nitrogen fertilizer (full rate N), and a control plot. Figure 2.2 shows plot maps for hay and grazed location. Ammonium sulfate (21-0-0-24(S), Harrell's, Lakeland, FL) was the fertilizer added to treatments including nitrogen fertilizer applications. At grazed locations, the full rate of nitrogen fertilizer applied was 84 kg N/ha (75 lb N/acre) and the half rate was 42 kg N/ha (37.5 lb N/acre). At hay locations, the full rate of nitrogen fertilizer applied was 112 kg N/ha (100 lb N/acre) and the half rate was 56 kg N/ha (50 lb N/acre). These nitrogen rates were based on recommendations set-forth by the Alabama extension cooperative (Dillard et al. 2020). Treatments were applied two times per year, with the initial application occurring in May and the second application occurring 8-12 weeks after initial application (late July or early August).

Data Collection:

Samples were collected four times per year, with two harvests after each application. Harvests took place every 4-6 weeks after the initial application, with the second application occurring at immediately after the second harvest. Forage biomass data were collected at all six locations to determine if differences between treatments were affected by forage management practices. This also allows us to evaluate PGPR treatment under varying field conditions.

Arthropod populations were surveyed taken at the three sites managed for hay production via sweep samples.

For forage biomass data, heights and weights were taken from each plot at every harvest. Forage from the entire 1 m^2 plot was cut by hand to an approximate height of 5 cm (2 inch) using mechanical hedge trimmers (Makita, Makita U.S.A., Inc.) and collected into individual bags. Forage was then dried in a forced air oven at 60°C for 48-72 hours until 90-95% dry matter was obtained. Forage dry matter (D.M.) was obtained after the drying process was completed to estimate forage biomass yield.

Sweep samples for arthropods were collected via ten sweeps per plot with a standard insect sweep net. Samples were then placed into individual bags and stored in a freezer until they were sorted and counted. Bermudagrass stem maggots (Diptera: Muscidae) and forage feeding caterpillars, including fall armyworms (Lepidoptera: Noctuidae) and grass loopers (Lepidoptera: Erebidae), were sorted and counted by hand. Other groups of arthropods counted include the families Acrididae, Tettigoniidae, Chrysomelidae, and the order Hemiptera.

Statistical Analysis:

Data were analyzed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC) for a completely randomized augmented factorial design. Orthogonal contrasts of treatment were used for mean comparisons. Forage biomass and arthropod populations were analyzed separately. For forage biomass samples, those collected from grazed locations and hay fields were analyzed separately as well. Location, year, and replicate were set as random variables and harvests were set as repeated measure. For forage biomass and arthropod populations, a significance level at $P \leq 0.05$ was declared.

Results:

Arthropod Populations:

Means for bermudagrass stem maggot (BSM) and forage feeding caterpillar counts were extremely low and yielded no significant difference between treatments (Figures 2.3 A and B). Acrididae and Tettigoniidae, plant feeding orthopterans, and Chrysomelidae (leaf beetles), primarily flea beetles and cucumber beetles, were also present in low numbers and yielded no significant difference between treatments (Figures 2.4A). Hemipterans (Figure 2.4B) were the most abundant and means indicated slightly higher counts in plots treated with DH44. However, these results yielded no statistical significance between treatments. Hemipteran populations are estimated to be comprised primarily of Cicadellidae, but also included small percentages of Cercopidae, Miridae, Lygaeidae, and Membracidae.

Forage Biomass:

Forage height at grazed locations were similar across all treatments, yielding no significance (Figure 2.5A). Forage biomass at grazed locations was greater in plots treated with nitrogen fertilizer and was the greatest in plots treated with full rates of nitrogen fertilizers (Figure 2.5B). A full rate of nitrogen fertilizer yielded higher forage biomass than that of the control ($P = 0.0005$). The biomass collected from control plots was not statistically different from biomass collected from DH44 plots ($P = 0.6266$) or B20 plots (P = 0.1473). However, plots treated with DH44 plus a half rate of nitrogen fertilizer ($P = 0.0216$) and B20 plus a half rate of nitrogen fertilizer ($P = 0.0264$) both yielded significantly greater forage biomass than that of the control plots. The addition of a half rate of fertilizer to DH44 did not significantly increase forage biomass relative to DH44 treatment alone ($P = 0.4381$). However, B20 treatments with half rates of nitrogen fertilizer yielded numerically greater biomass than those treatments of B20 alone (*P* = 0.0695). A full rate of nitrogen yielded higher weights than the plots treated with B20 (*P* = 0.0388) and DH44 (*P* = 0.0025). However, when comparing results from a full rate of nitrogen fertilizer to PGPR treatments including a half rate of nitrogen fertilizer, only minor differences were indicated (B20: *P* = 0.2239)(DH44: *P* = 0.1953).

Forage heights at hay locations were greater in plots treated with a full rate of nitrogen, and both plots treated with PGPR and a half rate of fertilizer (Figure 2.6A). Forage heights were lowest in plots treated with DH44, which yielded significantly lower than that of plots treated with a full rate of nitrogen fertilizer (*P* = 0.0176). Forage biomass at hay locations was greatest in plots treated with nitrogen fertilizer and were only slightly greater in plots treated with a full rate of nitrogen fertilizer (Figure 2.6B). Plots treated with a full rate of nitrogen fertilizer had greater biomass than those of the control (*P* = <.0001). Plots treated with PGPR alone yielded numerically greater forage biomass than the control, but no significant difference were determined (B20: *P* = 0.0536) (DH44: *P* = 0.1442). In comparison to the control plots, plots treated with B20 plus a half rate of nitrogen fertilizer (*P* = 0.0002) and plots treated with DH44 plus a half rate of nitrogen fertilizer (*P* = 0.0008) yielded significantly greater forage biomass. Plant growth promoting rhizobacteria treatments with nitrogen fertilizer produced greater forage biomass than plots treated with PGPR alone in both B20 plots (*P* = 0.0552) and DH44 plots (*P* = 0.0709). When comparing B20 treatments to DH44 treatments, forage biomass was similar (*P* = 0.5562). A full rate of nitrogen fertilizer yielded greater biomass than plots treated with only PGPR applications in B20 (*P* = 0.0143) and DH44 plots (*P* = 0.0035). However, comparisons of a full rate of nitrogen with PGPR treatments including a half rate indicate no significant differences in B20 plots (*P* =

0.3128), nor DH44 plots (*P* = 0.5160). *P*-values for forage heights and biomass at grazed and hay locations are listed on Table 2.2 with significant values highlighted. Forage biomass means in kg DM/ha for grazed and hay locations at listed on Table 2.3.

Discussion:

In this 2-year field experiment, PGPR and mixed PGPR and nitrogen treatments were evaluated on six producer fields in Alabama. These novel treatments were compared to the recommended rate of nitrogen fertilizer (Dillard et al. 2020). Effects on arthropod populations and forage biomass were evaluated to determine differences in hay fields between treatments. Forage arthropod samples yielded no significant differences between treatments. These results are consistent with previous studies conducted on fall armyworms and B20, in which B20 was determined to be non-biopesticidal (Coy et al. 2017). However, direct effects are not the only way in which PGPR can be utilized in pest management. Plant growth promoting rhizobacteria can also enhance stress tolerance to pests by stimulating root growth that enables bermudagrass to outgrow damage caused by pests (Coy et al. 2019, Coy et al.2020).

Our results of forage biomass analysis indicate that PGPR can yield greater biomass than control (untreated) plots. This is consistent with previous applications of PGPR on bermudagrass that resulted in significant increases in root and shoot biomass (Coy et al. 2014). No significant differences were observed when comparing forage biomass from plots treated with B20 and DH44. These results indicate that B20 and DH44 treatments can both enhance growth promotion in hybrid bermudagrass. Plant growth promoting rhizobacteria are biostimulants that enhance nutrient uptake, but do not physically add nutrients to the system (Calvo et al. 2014). This explains why PGPR treatments with a half rate of nitrogen fertilizer yielded higher biomass than plots treated with only PGPR. Furthermore, PGPR treatments that were applied with a half rate of nitrogen fertilizer yielded similar biomass as plots treated with a full rate of nitrogen fertilizer. This is consistent with a previous study on stockpiled bermudagrass in which these same PGPR treatments were applied. The results from this study also indicated that when applied with a half rate of nitrogen fertilizer, DH44 and B20 can yield similar results as the standard fertilizer rates (Griffin et al. 2020). The forage biomass in kg DM/ha reported in Griffin et al. 2020 were similar to dry biomass yields from this study. These treatments show great potential for reducing the amount of nitrogen fertilizer needed to optimize forage utilization in both grazed pastures and hay fields.

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Site Details						
Locations:	**Hay or Grazed:**	**Latitude:**	**Longitude:**	**Cultivar:**	**Soil Map Identification:**	**Soil Taxonomic Class:**
Wiregrass (Houston Co.)	Grazed	31.349	-85.319	Tifton 85	Dothan Fine Sandy Loam, 2 to 5 percent slopes	Fine-loamy, kaolinitic, thermic Plinthic Kandiudults
Wiregrass (Dale Co.)	Hay	31.4455	-85.6382	Russell	Lakeland Loamy Fine Sand, 0 to 5 percent slopes	Loamy, siliceous, thermic Grossarenic Kandiudults
Clay County	Grazed	33.3502	-85.7321	Russell	Madison Gravelly Sandy Loam, 6 to 10 percent slopes Madison-Riverview Association, Hillt	Fine, kaolinitic, thermic Typic Kanhapludults
Clay County	Hay	33.3429	-85.7284	Russell	Madison-Riverview Association, Hillt Madison Gravelly Sandy Loam, 6 to 10 percent slopes	Fine, kaolinitic, thermic Typic Kanhapludults
Macon County	Grazed	32.5311	-85.6711	Russell	Marvyn Loamy Sand 2 to 5 percent slopes	Fine-loamy, kaolinitic, thermic Typic Kanhapludults
Lawrence County	Hay	34.4312	-87.4909	Russell	Allen fine sandy loam, eroded, undulating phase	Fine-loamy, siliceous, semiactive, thermic Typic Paleudults

Table 2.1 Site details for hay and grazed sites including locations, management type, cultivar, soil map identification and soil taxonomic class. Soil identification and taxonomic class were reported based on NRSA web soil survey.

		Grazed		Hay	
	Orthogonal Contrasts	**Heights**	**Biomass**	**Heights**	**Biomass**
	-Control vs. +Control	*P* = 0.2507	**_P_ = 0.0005**	*P* = 0.0892	**_P_ = <.0001**
PGPR vs. -Control	-Control vs. B20	*P* = 0.9288	*P* = 0.1473	*P* = 0.3421	*P* = 0.0536
	-Control vs. DH44	*P* = 0.8495	*P* = 0.6266	*P* = 0.4973	*P* = 0.1442
	-Control vs. B20 + 1/2 Rate N	*P* = 0.4218	**_P_ = 0.0264**	*P* = 0.1442	**_P_ = 0.0002**
	-Control vs. DH44 + 1/2 Rate N	*P* = 0.3603	**_P_ = 0.0216**	*P* = 0.5173	**_P_ = 0.0008**
PGPR vs. PGPR	B20 vs. B20 + 1/2 Rate N	*P* = 0.4683	*P* = 0.0695	*P* = 0.1851	*P* = 0.0552
	DH44 vs. DH44 + 1/2 Rate N	*P* = 0.3721	*P* = 0.4381	*P* = 0.6088	*P* = 0.0709
	B20 vs. DH44	*P* = 0.7824	*P* = 0.5314	*P* = 0.0845	*P* = 0.5562
PGPR vs. +Control	+Control vs. B20	*P* = 0.2158	**_P_ = 0.0388**	*P* = 0.4521	**_P_ = 0.0143**
	+Control vs. DH44	*P* = 0.3374	**_P_ = 0.0025**	**_P_ = 0.0176**	**_P_ = 0.0035**
	+Control vs. B20 + 1/2 Rate N	*P* = 0.8147	*P* = 0.2239	*P* = 0.2917	*P* = 0.3128
	+Control vs. DH44 + 1/2 Rate N	*P* = 0.7293	*P* = 0.1953	*P* = 0.8101	*P* = 0.5160

Table 2.2 *P*-values from orthogonal contrasts of treatments on forage heights and biomass at grazed and hay locations in 2020 and 2021.

P-values of <0.05 are listed in bold pink text.

+Control = Full rate of nitrogen. -Control = non-treated plots.

	Grazed		Hay	
Treatments	**Mean Biomass**	**SEM**	**Mean Biomass**	**SEM**
DH44	1328.35 kg DM/ha	216.00	1336.99 kg DM/ha	89.90
DH44 + ½ Rate of N	1443.49 kg DM/ha	149.36	1574.54 kg DM/ha	87.92
B20	1185.15 kg DM/ha	212.69	1275.03 kg DM/ha	105.82
B20 + ½ Rate of N	1455.24 kg DM/ha	138.42	1527.25 kg DM/ha	92.33
Full Rate of N	1635.95 kg DM/ha	187.82	1659.81 kg DM/ha	130.59
Control	1112.92 kg DM/ha	197.22	1083.12 kg DM/ha	117.61

Table 2.3 Mean biomass values and SEM across all grazed and hay locations in 2020 and 2021.

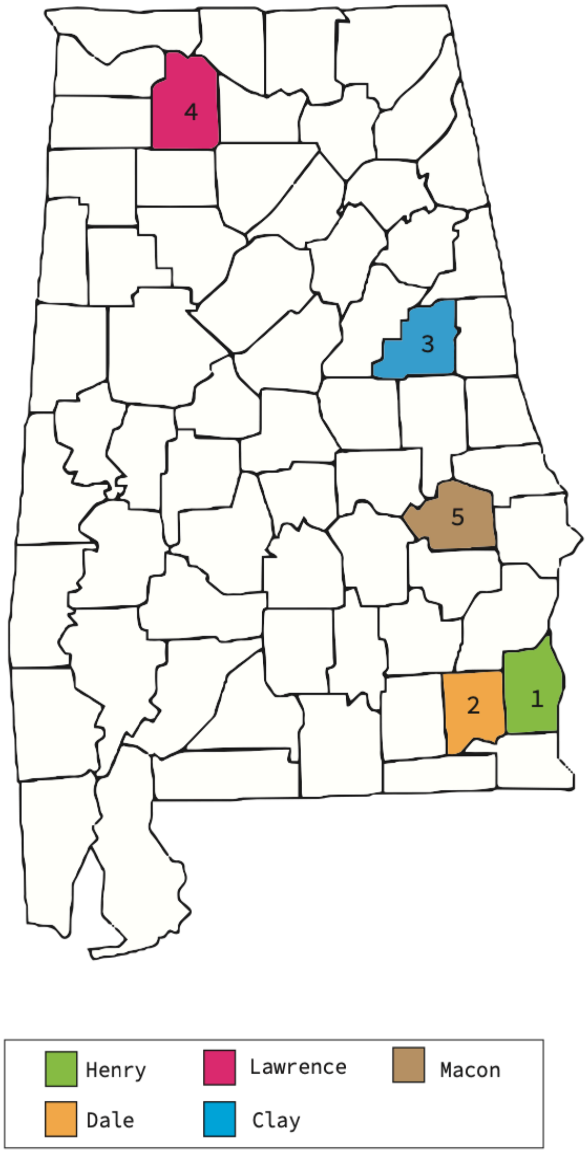
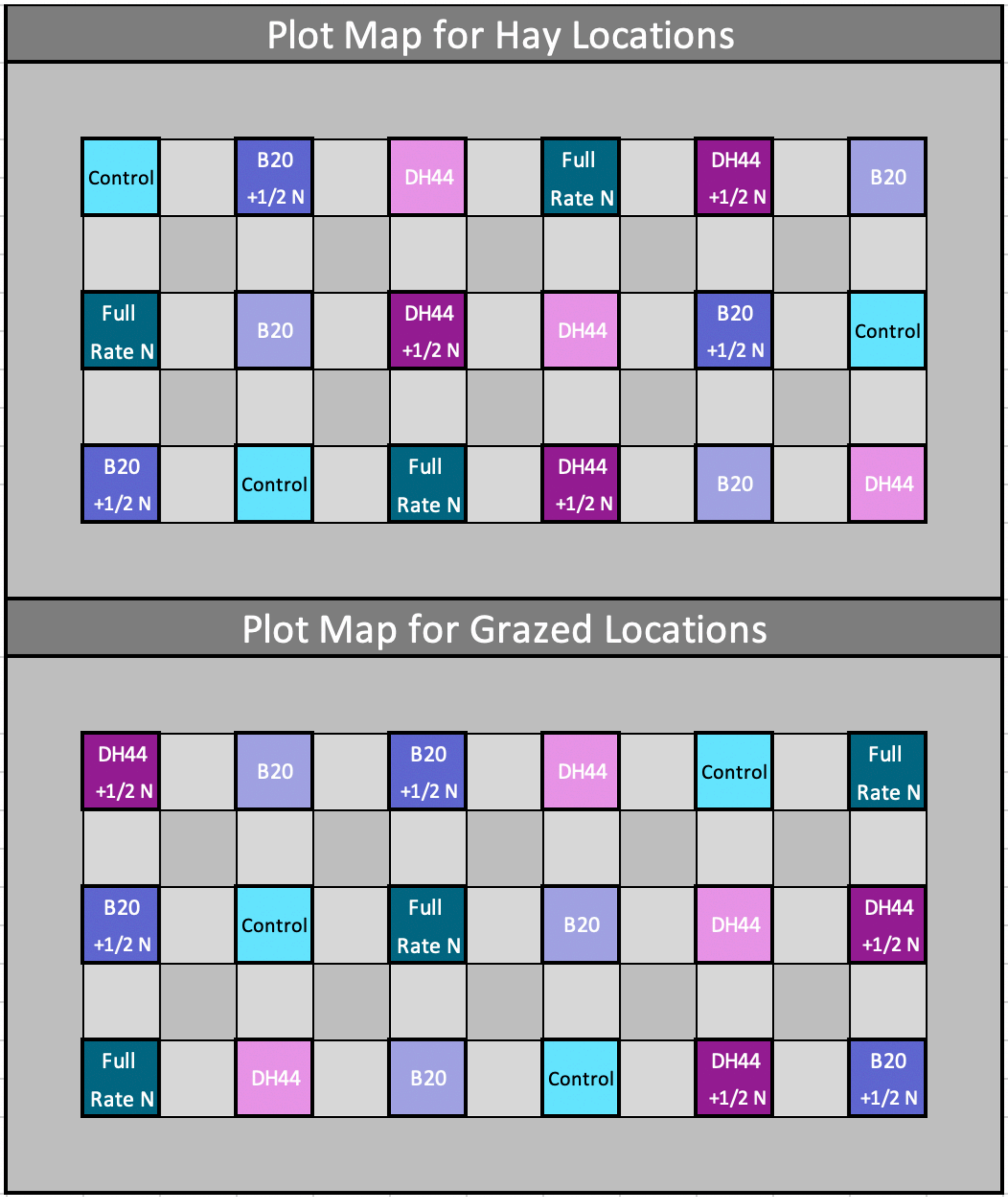


Figure 2.1 Alabama state map with counties of sites highlighted.
Hay sites were located in Dale County, Clay County, and Lawrence County (2, 3, 4).
Grazed sites were located in Henry County, Clay County, and Macon County (1, 3, 5).



Plot Map for Hay Locations

Control	B20 +1/2 N	DH44	Full Rate N	DH44 +1/2 N	B20
Full Rate N	B20	DH44 +1/2 N	DH44	B20 +1/2 N	Control
B20 +1/2 N	Control	Full Rate N	DH44 +1/2 N	B20	DH44

Plot Map for Grazed Locations

DH44 +1/2 N	B20	B20 +1/2 N	DH44	Control	Full Rate N
B20 +1/2 N	Control	Full Rate N	B20	DH44	DH44 +1/2 N
Full Rate N	DH44	B20	Control	DH44 +1/2 N	B20 +1/2 N

Figure 2.2 Plot map and treatment layout of hay and grazed locations.

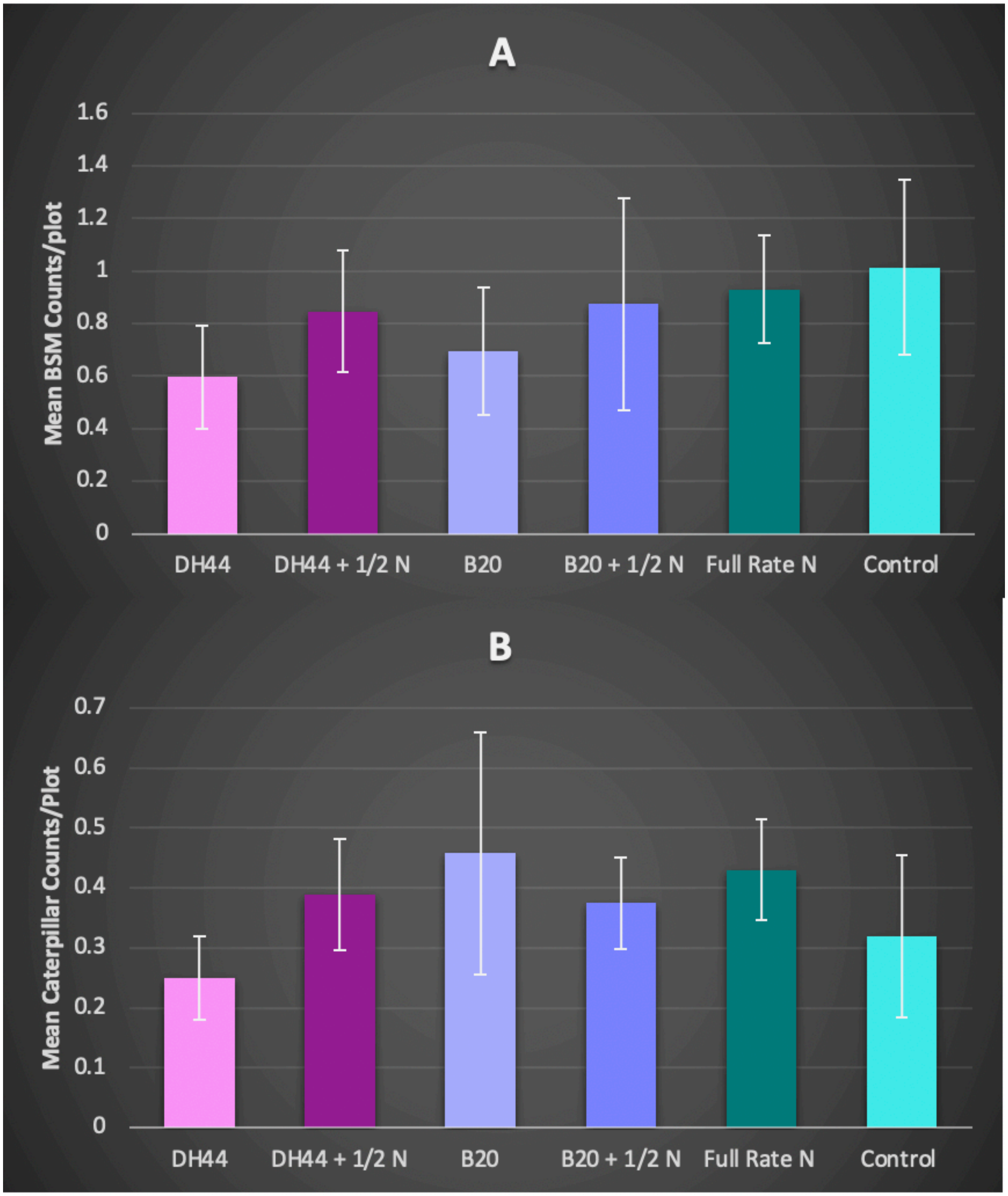


Figure 2.3 Mean counts of bermudagrass stem maggots (BSM) and forage feeding caterpillars across all three hay locations in 2020 and 2021.
A.) includes mean BSM counts per plot
B.) includes mean forage feeding caterpillar counts per plot.
Plots were 1 m^2 in size and samples were collected with 10 sweeps with a sweep net.
Full Rate N = + Control
Control = -Control/Non-Treated

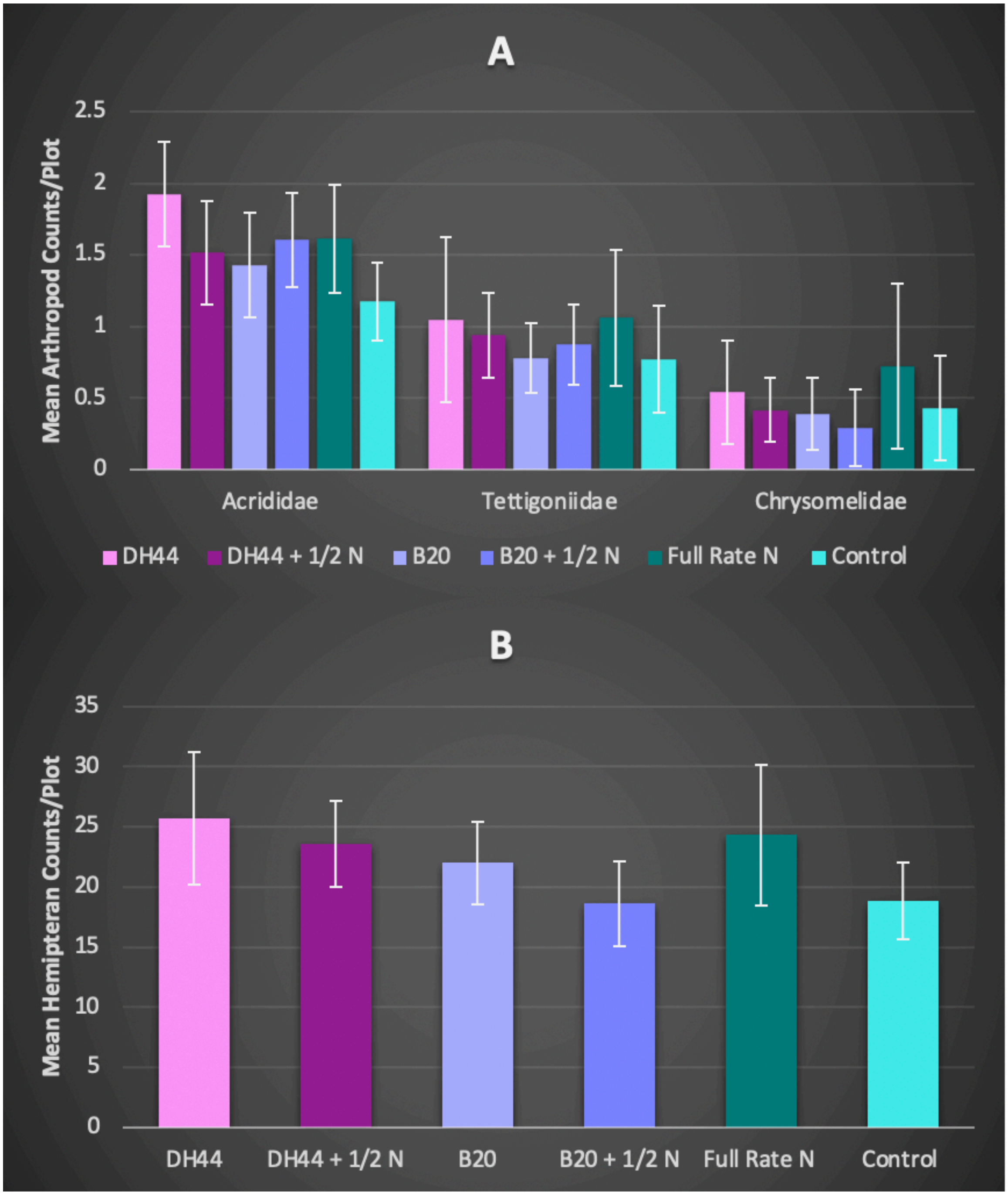


Figure 2.4 Mean counts of arthropods across all three hay locations in 2020 and 2021.
A.) Includes counts of Orthoptera (Acrididae & Tettigoniidae) and Coleoptera: Chrysomelidae per plot
B.) Includes mean Hemipteran counts per plot.
Plots were 1m^2 in size and samples were collected with 10 sweeps with a sweep net.
Full Rate N = + Control
Control = -Control/Non-Treated

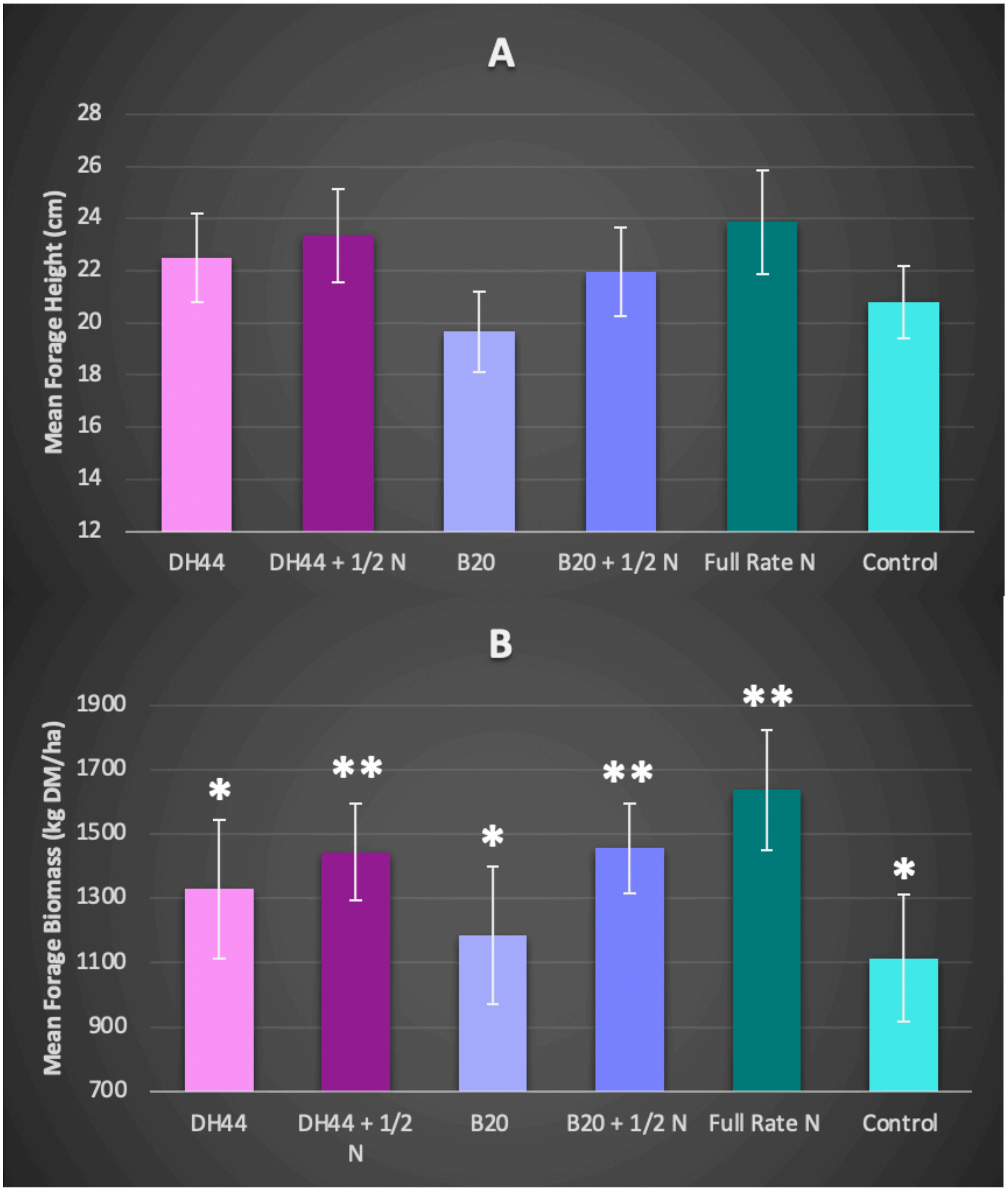


Figure 2.5 Mean forage heights and DM yields for grazed locations in 2020 and 2021.
A. Mean forage heights in cm at grazed locations
B. Mean forage biomass in kg DM/ha at grazed locations
* Indicates statistically greater than control ($P < 0.05$)
** indicates statistically less than full rate of N ($P < 0.05$)
Full Rate N = + Control
Control = -Control/Non-Treated

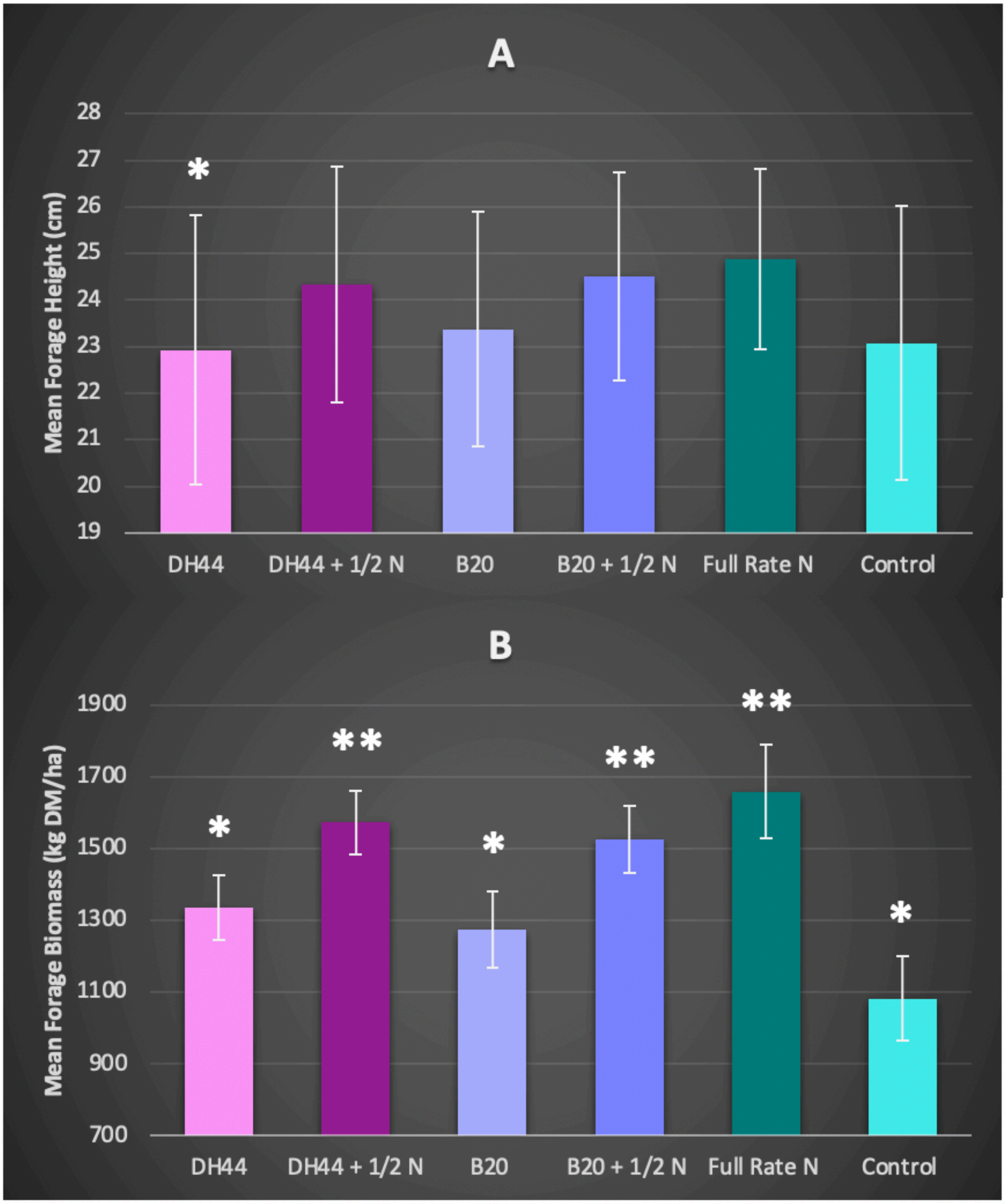


Figure 2.6 Mean forage heights and DM yields for hay locations in 2020 and 2021.
A. Mean forage heights in cm at hay locations
B. Mean forage biomass in kg DM/ha at hay locations
* Indicates statistically greater than control ($P < 0.05$)
** indicates statistically less than full rate of N ($P < 0.05$)
Full Rate N = + Control
Control = -Control/Non-Treated

Chapter III:

Effects of Plant-Growth Promoting Rhizobacteria and Nitrogen Fertilizer on Forage Growth and Soil Health in Bermudagrass Fields

Introduction:

Maintaining a healthy soil biota is vital for plants and animals in the surrounding ecosystems (Nelson et al. 2020). Essential ecosystem services provided by the soil biota include decomposition of organic matter, nutrient cycling, and pest suppression with soil microbes (e.g., bacteria and fungi) and mesofauna (e.g., collembola and mites) being key players in these roles (Neher and Barbercheck 2019). Soil microbes and mesofauna are sensitive to changes in the soil, so management and land-use can significantly impact these populations and the ecosystem services they provide (Yao et al. 2006). The application of nitrogen fertilizers has been shown to create cytotoxic areas in the soil resulting in microbial dead zones and decreased populations of mesofauna (Ruiz et al. 2020, Lohm et al. 1977). Soil mesofauna and microbes are vital components of soil health and populations must be preserved to maintain the health of the surrounding ecosystems (Neher and Barbercheck 2019). As loss of ecosystems services become more apparent, the need to incorporate environmental based rational into chemical applications is more important than ever (Costanza et al. 2017).

Exploring biological alternatives that result in sustainable agriculture that is ecological sound is essential (Sanderson and Liebig 2020). Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria that promote plant growth through several mechanisms (Prasad et al. 2019). Use of PGPR as a biostimulant has displayed promising potential for reducing chemical fertilizer applications in bermudagrass and the agricultural industry (Adesemoye et al. 2009).

Previous studies conducted at Auburn University have identified several strains of PGPR that are effective at growth promotion in hybrid bermudagrasses (*Cynodon dactylon* (L.) Pers). Bermudagrass is a warm-season perennial grass in the southeastern United States. Hybrid bermudagrass cultivars are commonly utilized in hay production and grazing systems due to their drought tolerance and pronounced yield response to nitrogen fertilizer (Lemus 2018). Inoculation with blends of *Bacillus* spp. have resulted in significant increases in both root and shoot biomass of bermudagrass in greenhouse trials (Coy et al. 2014). After initial colonization, strains of PGPR can persist in bermudagrass roots and shoots for 8-12 weeks (Coy et al. 2019). With these results in mind, the goal of this study was to evaluate the potential of PGPR to reduce chemical fertilizer rates needed in forage production by comparing PGPR treatments to treatments of nitrogen fertilizer. In particular, the objectives of this study were to (1) compare the effects of PGPR and nitrogen fertilizers on forage biomass in bermudagrass fields utilized for hay production and grazing, (2) compare the effects of PGPR and nitrogen fertilizers on arthropod populations in bermudagrass hay fields, and (3) compare effects of PGPR and nitrogen fertilizers on soil health.

Materials and Methods:

PGPR Strains:

For this study, a blend of three *Bacillus* strains, known as Blend 20, and a single strain application of *Paenibacillus* sp., were applied as the PGPR treatments. Blend 20 consists of two strains of *Bacillus pumilus* (AP7 and AP18), and a strain of *Bacillus sphaericus* (AP282). The single strain application of *Paenibacillus* sp. DH44, a species closely related to *Paenibacillus sonchi*. These bacteria were grown in the lab and scraped into solution for field applications. All strains were grown in an incubator at 28°C for their optimal growth dates. AP282, *Bacillus sphaericus*, was allowed to grow for three days before scraping. AP7 and AP18, *Bacillus pumilus*, were grown for 5 days. And DH44, *Paenibacillus* spp., was grown for 10-12 days before scraping. Upon the optimal growth date, the bacteria were scraped into solution using autoclaved de-ionized water. After being scraped into 250mL bottles, the PGPR were soaked in a water bath at 80°C to kill off the vegetative phase and leave the endospores for field applications. Blend 20 was applied at a concentration of 1×10^7 and DH44 was applied at a concentration of 5×10^6.

Field Evaluation:

A field experiment was conducted from May through October of 2020 at two sites at the EV Smith Research Unit in Macon County, Alabama. These sites had established stands of Tifton 85 bermudagrass utilized for hay production. The coordinates for the field in which these sites were located are 32.4245, -85.8954. The soil type at the first site was a Compass loamy sand with a taxonomic classification of coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults. While site two had a Luverne sandy loam taxonomically classified as fine, mixed, semiactive, thermic, Typic Hapludults. These soil types and classes were as specified by the NRSA web soil survey.

The experimental design of this project was an augmented factorial randomized block design with six treatments in 3 m^2 plots with three replicates per site. The six treatments included Blend 20 (B20), B20 plus a half rate of nitrogen fertilizer (B20 + ½ N), DH44, DH44 plus a half rate of nitrogen fertilizer (DH44 + ½ N), a full rate of nitrogen fertilizer (full rate N), and a control plot. Figure 3.1 includes plot maps for these sites. Ammonium sulfate (21-0-0-24(S), Harrell's, Lakeland, FL) was used for nitrogen fertilizer applications. The full rate of nitrogen fertilizer applied was 112 kg N/ha (100 lb N/acre) and the half rate was 56 kg N/ha (50 lb N/acre). These nitrogen rates were based on recommendations set-forth by the Alabama extension cooperative (Dillard et al. 2020). Treatments were applied two times per year, with the initial application occurring in May and the second application occurring 8-12 weeks after initial application (late July or early August).

Data Collection:

Forage and sweep samples were collected four times per year, with two harvests after each application. Harvests took place every 4-6 weeks after the initial application, with the second application occurring at immediately after the second harvest. Forage samples and sweep samples were collected at all four harvests. Soil samples were collected at the third harvest in September of 2020 to analyze soil health factors.

Forage heights and weights were taken from each plot at every harvest. Six, 0.3 m (1 ft) quadrats were placed at random in each 3 m^2 plot and forage was cut by hand to an approximate height of 5 cm (2 inch) using mechanical hedge trimmers (Makita, Makita U.S.A., Inc.). Forage biomass clippings were then collected into individual bags and placed in a forced air oven for 48-72 hours at 60°C. Forage dry weights were obtained after the drying process was completed to estimate forage biomass yield.

Sweep samples for arthropod samples were collected via 20 sweeps per plot with a standard insect sweep net. Samples were then placed into individual bags and stored in a freezer until they were ready to be sorted and counted. Bermudagrass stem maggots (Diptera: Muscidae) and forage feeding caterpillars, including fall armyworms (Lepidoptera: Noctuidae) and grass loopers (Lepidoptera: Erebidae), were sorted and counted by hand. Other groups of arthropods counted include Acrididae, Tettigoniidae, Chrysomelidae, and Hemiptera.

Soil samples were collected using Par Aide HiO^{TM} hole cutters with outside sharpened blades (1003-2, Par Aide, St. Paul, Minnesota). Two soil cores, 11.7 cm (4.6 inch) in diameter, were taken from each 3 m^2 plot, bagged into plastic bags, then placed into a cooler for transport to the lab (Kunkel et al. 1999). In the lab, soil cores were place in a Tullgren funnel system for 48-72 hours under incandescent light to extract the soil arthropods. Glass jars with ethanol were placed under each funnel to collect mesofauna as the soil dried. After the soil was completely dry, mesofauna samples were collected into 50 mL centrifuge tubes and stored in the refrigerator until they were ready to be counted. Soil mesofauna samples were counted under a microscope focusing on indicators of soil health. Collembola and three types of mites were identified and counted in each sample. The three types of mites included prostigmatids, which are predatory mites, mesostigmatids and oribatids, which are both important in the decomposition of organic matter. Soil from each funnel was sieved, weighed out to 100 g, and analyzed for soil respiration at a commercial laboratory (Wade laboratories, Kearney, NE). Their procedure, in brief, used a drying and re-wetting technique to collect the CO_2 – C ratio in ppm of C (Culman 2019).

Statistical Analysis:

Data were analyzed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC) for a completely randomized augmented factorial design. Orthogonal contrasts of treatment were used for mean comparisons. Forage biomass and arthropod populations were analyzed separately. Location, year, and replicate were set as random variables and harvests were set as a repeated measure. For forge biomass and arthropod populations, a significance level at $P \leq 0.05$ was declared.

Results:

Arthropod Populations:

Mean counts of bermudagrass stem maggot flies were highest in plots treated with B20 and B20 + ½ N (11.2); however, no treatment was statistically different from one another or the controls (Figure 3.2A). Forage feeding caterpillar counts were extremely low and no significant differences in treatments was found (Figure 3.2B). Mean counts of Acridids, Tettigonids, and Chrysomelids were also low and did not yield significance (Figure 3.3A). Hemipteran counts were the largest amongst arthropod groups, but no significant differences were seen between treatments (Figure 3.3B). Hemipteran populations are estimated to be comprised primarily of Cicadellidae, but also included small percentages of Cercopidae, Miridae, Lygaeidae, and Membracidae.

Forage Biomass:

At these sites, forage heights were similar across all treatments and there were no statistically significant differences in yield (Figure 3.4A). Forage DM yield was greater in plots treated with DH44 plus a half rate of nitrogen fertilizer and plots treated with a full rate of nitrogen fertilizer, with DH44 plus a half rate of nitrogen fertilizer being the greatest (Figure 3.4B). Although these treatments are not statistically significant based on the set value ($P > 0.05$), they may be considered biologically significant. In comparison to the control, plots treated with a full rate of nitrogen yielded numerically greater weights ($P = 0.0918$). And plots treated with DH44 plus a half rated yielded more forage weight in comparison to the control plots ($P = 0.0555$). *P*-values of orthogonal contrasts are listed Table 3.1.

Soil Respiration:

Soil respiration as reported as CO_2-C ratios (ppm C) were greatest in the control plots. Plots treated with B20 and DH44 also yielded slightly greater CO_2-C ratios than that of plots treated with nitrogen fertilizer (Figure 3.5). However, these trends were not statistically significant.

Soil Mesofauna:

The evaluated groups of mesofauna varied widely but seemed to have an overall trend of higher populations in plots that were not treated with nitrogen fertilizers (Figure 3.6). Prostigmatid mites were too low to identify trends between treatments. Collembolans counts were also low, but there were numerically more in PGPR and control plots than in those including a nitrogen fertilizer application. Populations of mesostigmatid and oribatid mites were generally greater than those of collembolans and prostigmatid mites, with oribatid mites having the greatest numbers. Trends of oribatid counts show highest populations in plots with B20 and DH44 alone. While mesostigmatids have highest counts in the PGPR plots as well as the plots treated with B20 and nitrogen fertilizer. Statistical analyses were limited, and no significance was identified in populations of prostigmatids, mesostigmatids, and collembolans. However, populations of oribatids were significantly higher in plots treatment with PGPR alone than in plots treated with a full rate of nitrogen fertilizer ($P = 0.0027$) and control plots ($P = 0.0076$) (Table 3.2).

Discussion:

In this one-year study, the effects of PGPR with and without nitrogen fertilizer application were evaluated on forage biomass, arthropod populations, and aspects of soil health. These effects were compared to those of a full rate of nitrogen fertilizer and a control with no treatments. Data from these plots were collected through November 2021 but are not presented here. Arthropod populations in 2020 were not affected by treatments. This lack of treatment effect is partially due to low insect counts and is consistent with previous studies. Coy et al. (2017) conducted greenhouse experiments showing no negative impact of B20 on larval development of fall armyworm. The authors did report impacts on egg laying choice and overall fecundity. It was feasible that those effects on oviposition may have reduced populations of forage feeding pests but that was not observed.

Forage biomass in this one-year study did not yield any statistical significance even when comparing full rate of N to control plots. However, treatments of DH44 plus a half rate of nitrogen and a full rate of nitrogen fertilizer did yield numerically greater forage biomass weights than that of the control plots. A field study on stockpiled bermudagrass indicated that, when applied with half of the recommended rate of nitrogen fertilizer, Blend 20 and DH44 yielded similar forage biomass and nutritional value as a full rate of nitrogen fertilizer (Griffin et al. 2020). The present study also reported greater biomass yields across all PGPR than those collected at both EV Smith sites in 2020. For example, Griffin et al. (2020) reported 1544 kg DM/ha in DH44 plots treated with a half rate of nitrogen fertilizer over a two-year period whereas, EV Smith sites only yielded 1003 kg DM/ha for year one with this same treatment. Data collected from the second year of this study may very well indicate similar results as the previous two-year study. However, the control and full rate of nitrogen plots at EV Smith also yielded lower forage biomass than those reported by Griffin et al. (2020) as well. Since the full rate of nitrogen in addition to the PGPR treatments did not yield significantly higher biomass than that of the control plots, this indicates an outside factor is at play. Griffin et al. (2020)

reported mean forage biomass yields of 1271 – 1914 kg DM/ha, while the forage biomass from EV Smith ranged from 884 – 1002 kg DM/ha. This indicates lower than average forage biomass yields within this study in regard to previous studies (Griffin et al. 2020). This could have been a result of several environmental factors, but the second year of this data will allow a better understanding the cause. In addition to forage biomass, forage quality may also be affected by PGPR treatments. Analysis of the forage quality was not yet completed but may provide additional insights into the relative value of PGPR for forage production.

Soil respiration trends indicate slightly higher soil microbial activity within plots treated with PGPR and control plots. This data was only collected at one harvest during September of 2020, therefore statistical analysis could not indicate differences due to a low number of replications. A second year of these samples were collected and will be analyzed for CO_2-C ratios (ppm C). Higher CO_2-C ratios indicate increased levels of soil microbial activity. As previously stated, soil microbes are responsible for many essential ecosystem services (Nelson et al. 2020). This increase in soil health will result in healthier ecosystems in the surrounding areas, particularly the plants growing within that soil (Neher and Barbercheck 2019). Previous studies linking microbial dead zones to nitrogen applications indicate that soil respiration could be significantly affected by nitrogen over time (Ruiz et al. 2020). Therefore, it is likely that these trends in decreased soil respiration in plots treated with nitrogen will yield statistical significance from the second year of data collection.

In terrestrial ecosystems, soil is one of the less studied resources. In particular, there is a lack of research regarding soil mesofauna, or microarthropods, with an estimate of less than 10% of soil species having been described (Ojeda and Gasca-Pineda 2019). Despite the lack of research, soil mesofauna are vital components of soil health and populations must be preserved to maintain the health of the surrounding ecosystems (Neher and Barbercheck 2019). The trends identified in this one-year study in bermudagrass are consistent with previous studies that linked decreases in mesofauna to fertilizer applications (Lohm et al. 1977). The results in this study were only collected at the third harvest in October of 2020 making our dataset less robust and statistical significance more diffucut to determine. However, oribatid counts from plots treated with PGPR alone yielded significantly higher rates than that of plots treated with a full rate of nitrogen fertilizer and control plots. These results indicate that mesofauna could be positively influenced by applications of PGPR. Counts of collembolans, mesostigmatids, and prostigmatids were lower than that of oribatids, and statistical analysis did not yield any significance between treatments. A second year of this data was collected and will be added to this data set. Considering the trends in population counts amongst treatments and the significance identified in oribatids, it seems likely that the second year of this study will yield statistically significant results in all four mesofauna groups. These results will help broaden our understanding of benefits provided by these PGPR strains as a result of decreasing nitrogen fertilizer applications.

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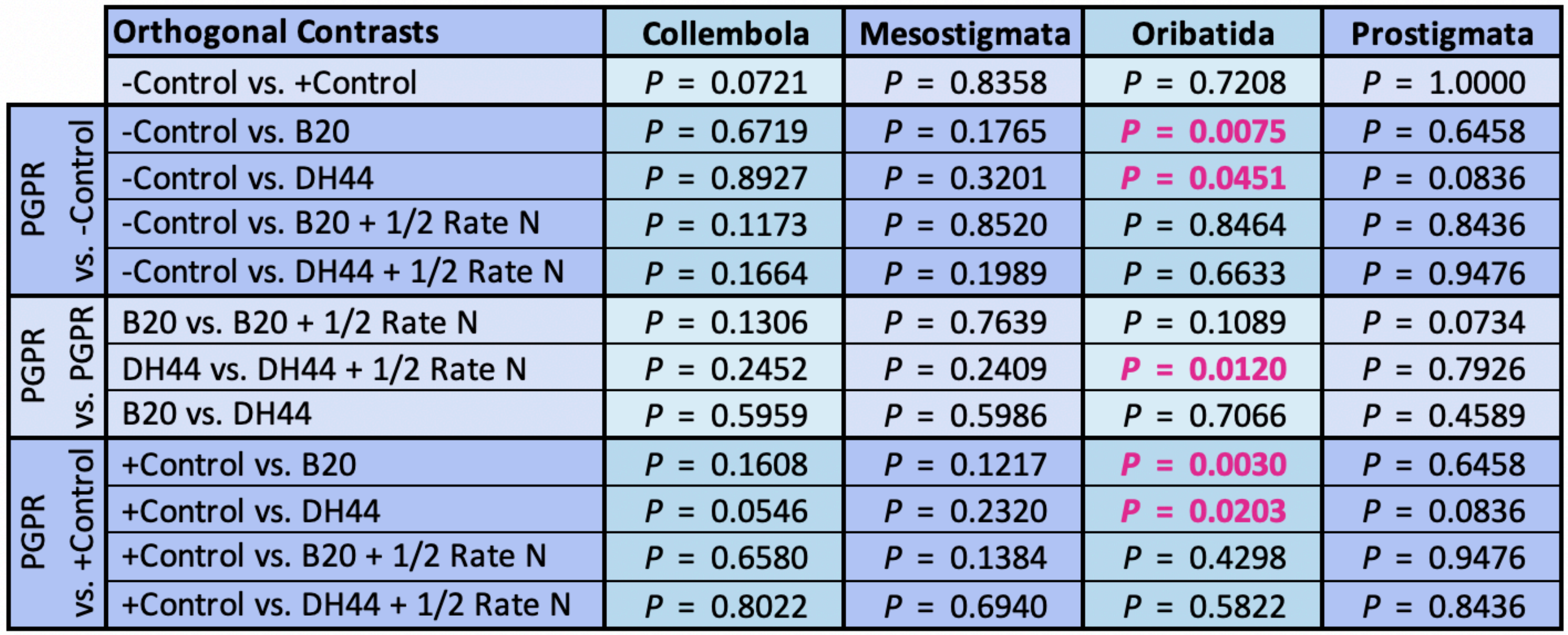
	Orthogonal Contrasts	Heights	Biomass
	-Control vs. +Control	*P* = 0.5139	*P* = 0.0918
PGPR vs. -Control	-Control vs. B20	*P* = 0.7288	*P* = 0.6211
	-Control vs. DH44	*P* = 0.5349	*P* = 0.8017
	-Control vs. B20 + 1/2 Rate N	*P* = 0.3545	*P* = 0.2940
	-Control vs. DH44 + 1/2 Rate N	*P* = 0.6809	*P* = 0.0555
PGPR vs. PGPR	B20 vs. B20 + 1/2 Rate N	*P* = 0.8339	*P* = 0.4239
	DH44 vs. DH44 + 1/2 Rate N	*P* = 0.2041	*P* = 0.1529
	B20 vs. DH44	*P* = 0.2553	*P* = 0.4283
PGPR vs. +Control	+Control vs. B20	*P* = 0.7593	*P* = 0.2309
	+Control vs. DH44	*P* = 0.9743	*P* = 0.1502
	+Control vs. B20 + 1/2 Rate N	*P* = 0.8088	*P* = 0.5197
	+Control vs. DH44 + 1/2 Rate N	*P* = 0.1158	*P* = 0.8151

Table 3.1 *P*-values from orthogonal contrasts of treatments on forage heights and biomass at both EV Smith sites in 2020.
+Control = Full rate of nitrogen. -Control = non-treated plots.

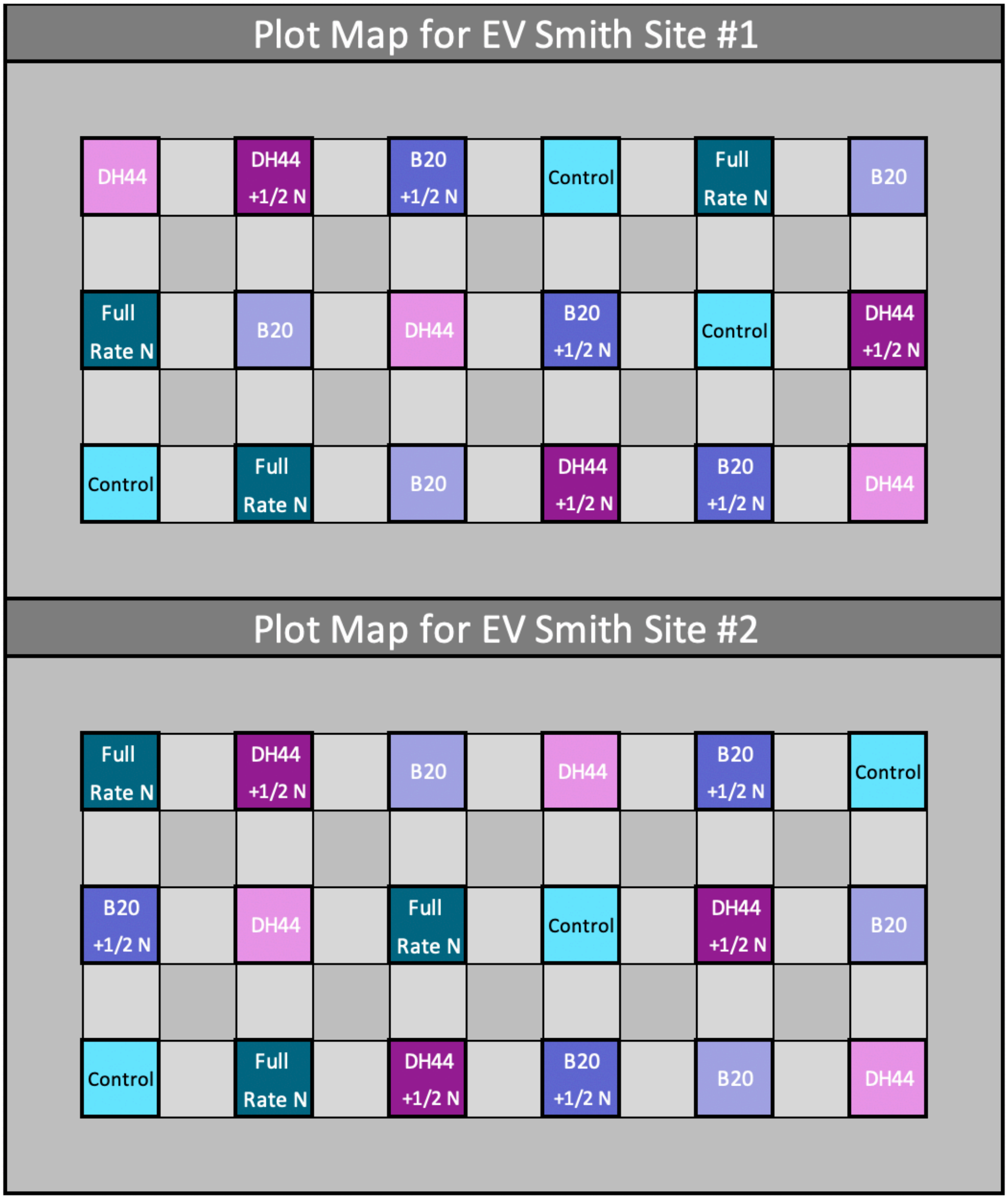


	Orthogonal Contrasts	Collembola	Mesostigmata	Oribatida	Prostigmata
	-Control vs. +Control	*P* = 0.0721	*P* = 0.8358	*P* = 0.7208	*P* = 1.0000
PGPR vs. -Control	-Control vs. B20	*P* = 0.6719	*P* = 0.1765	***P* = 0.0075**	*P* = 0.6458
	-Control vs. DH44	*P* = 0.8927	*P* = 0.3201	***P* = 0.0451**	*P* = 0.0836
	-Control vs. B20 + 1/2 Rate N	*P* = 0.1173	*P* = 0.8520	*P* = 0.8464	*P* = 0.8436
	-Control vs. DH44 + 1/2 Rate N	*P* = 0.1664	*P* = 0.1989	*P* = 0.6633	*P* = 0.9476
PGPR vs. PGPR	B20 vs. B20 + 1/2 Rate N	*P* = 0.1306	*P* = 0.7639	*P* = 0.1089	*P* = 0.0734
	DH44 vs. DH44 + 1/2 Rate N	*P* = 0.2452	*P* = 0.2409	***P* = 0.0120**	*P* = 0.7926
	B20 vs. DH44	*P* = 0.5959	*P* = 0.5986	*P* = 0.7066	*P* = 0.4589
PGPR vs. +Control	+Control vs. B20	*P* = 0.1608	*P* = 0.1217	***P* = 0.0030**	*P* = 0.6458
	+Control vs. DH44	*P* = 0.0546	*P* = 0.2320	***P* = 0.0203**	*P* = 0.0836
	+Control vs. B20 + 1/2 Rate N	*P* = 0.6580	*P* = 0.1384	*P* = 0.4298	*P* = 0.9476
	+Control vs. DH44 + 1/2 Rate N	*P* = 0.8022	*P* = 0.6940	*P* = 0.5822	*P* = 0.8436

Table 3.2 *P*-values from orthogonal contrasts of treatments on mesofauna at both EV Smith sites in 2020.
P-values of <0.05 are highlighted in pink.
+Control = Full rate of nitrogen. -Control = non-treated plots.

Treatments	Mean Biomass	SEM
DH44	901.79 kg DM/ha	37.29
DH44 + ½ Rate of N	1002.79 kg DM/ha	8.88
B20	884.66 kg DM/ha	33.42
B20 + ½ Rate of N	941.00 kg DM/ha	38.83
Full Rate of N	986.33 kg DM/ha	19.5
Control	867.00 kg DM/ha	20.5

Table 3.3 Mean biomass values for year one at both EV Smith sites in 2020.



Plot Map for EV Smith Site #1

DH44	DH44 +1/2 N	B20 +1/2 N	Control	Full Rate N	B20
Full Rate N	B20	DH44	B20 +1/2 N	Control	DH44 +1/2 N
Control	Full Rate N	B20	DH44 +1/2 N	B20 +1/2 N	DH44

Plot Map for EV Smith Site #2

Full Rate N	DH44 +1/2 N	B20	DH44	B20 +1/2 N	Control
B20 +1/2 N	DH44	Full Rate N	Control	DH44 +1/2 N	B20
Control	Full Rate N	DH44 +1/2 N	B20 +1/2 N	B20	DH44

Figure 3.1 Plot maps and treatment layout of EV Smith Site #1 and Site #2

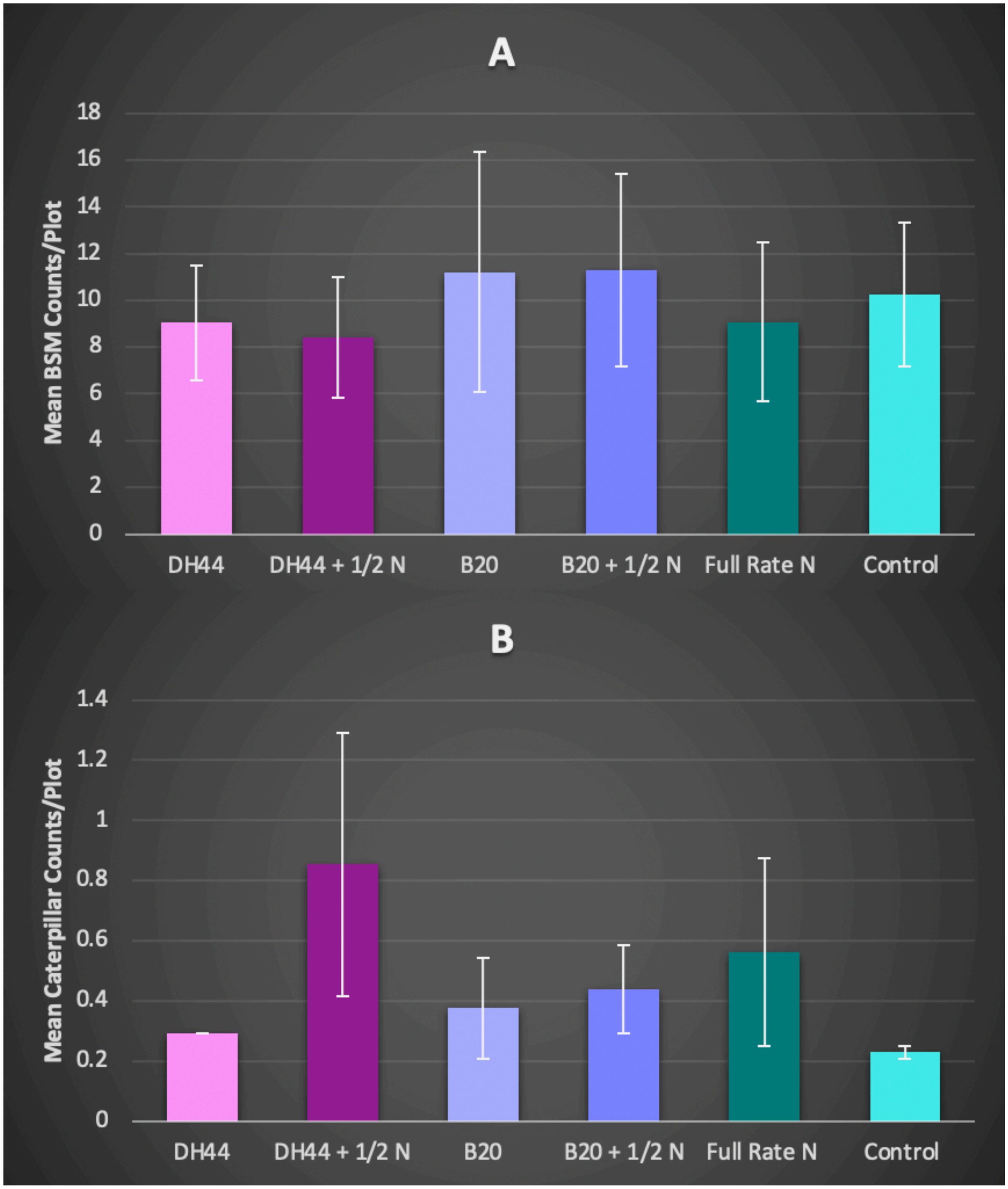


Figure 3.2 Mean counts of bermudagrass stem maggots (BSM) and forage feeding caterpillars across both EV Smith sites in 2020.
A.) includes mean BSM counts per plot
B.) includes mean forage feeding caterpillar counts per plot.
Plots were 3 m^2 in size and samples were collected with 20 sweeps with a sweep net.
Full Rate N = + Control
Control = -Control/Non-Treated

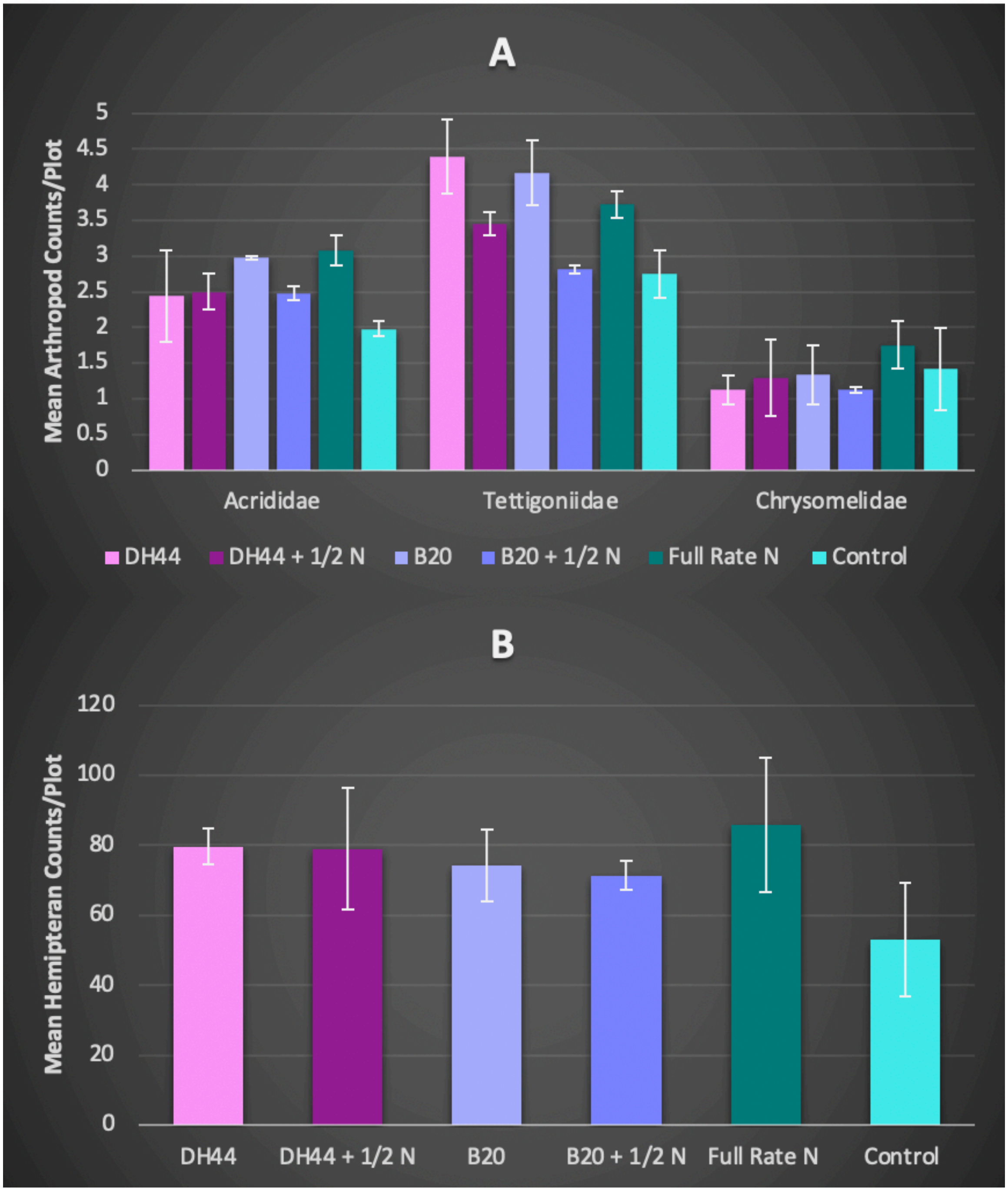


Figure 3.3 Mean counts of arthropods across both sites at EV Smith in 2020.
A.) Includes counts of Orthoptera (Acrididae & Tettigoniidae) and Coleoptera: Chrysomelidae per plot
B.) Includes mean Hemipteran counts per plot.
Plots were 3m^2 in size and samples were collected with 20 sweeps with a sweep net.
Full Rate N = + Control
Control = -Control/Non-Treated

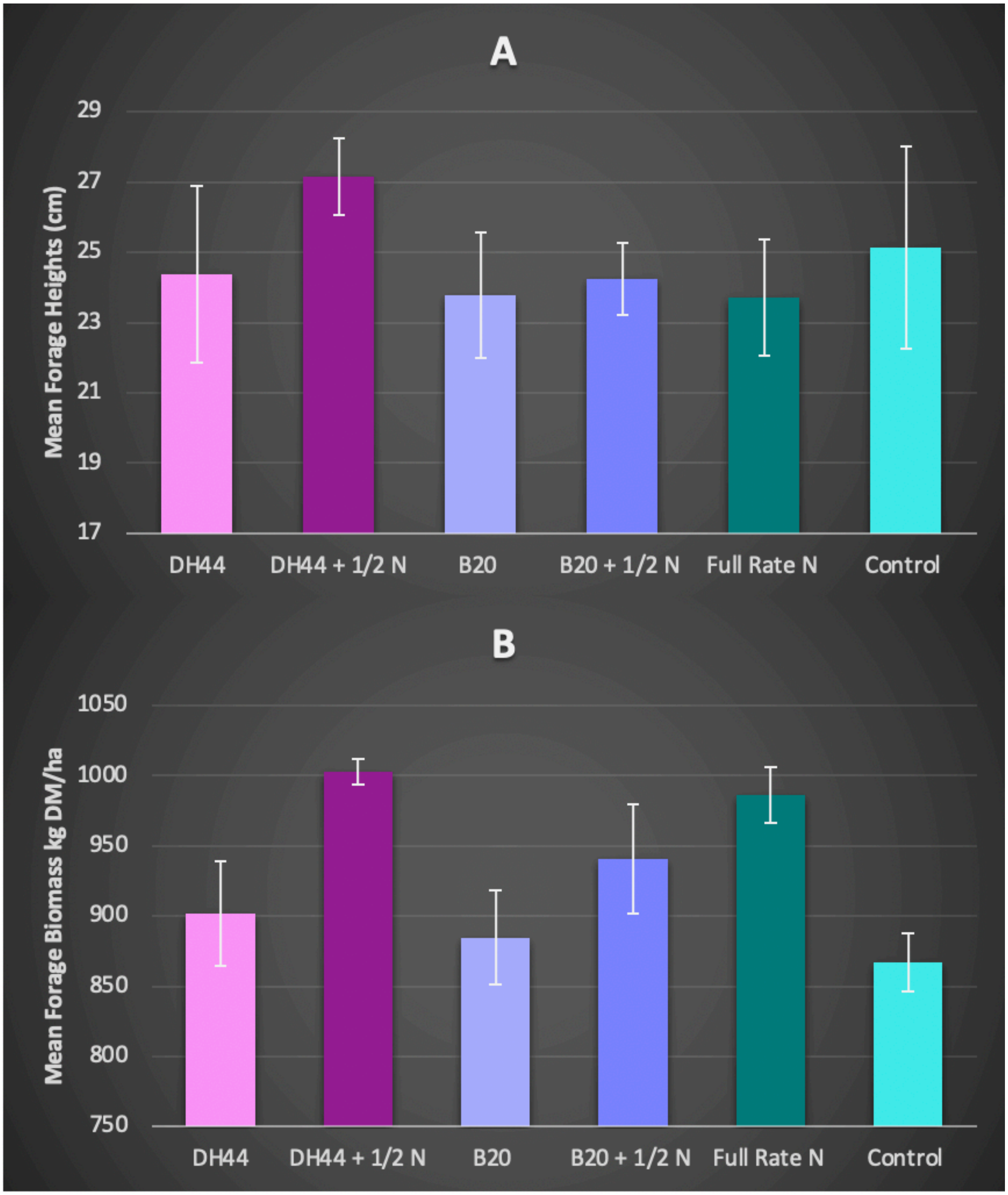


Figure 3.4 Mean forage heights and DM yields for both sites at EV Smith in 2020.
A. Mean forage heights in cm at hay locations
B. Mean forage biomass in kg DM/ha at hay locations
Full Rate N = + Control
Control = -Control/Non-Treated

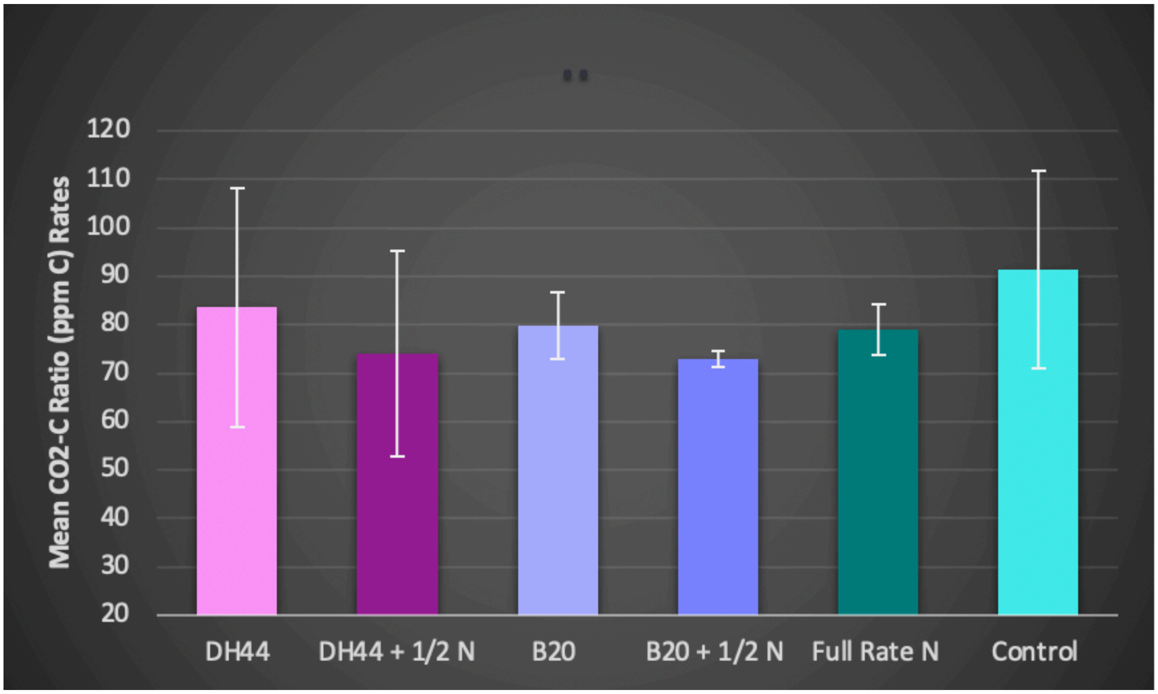


Figure 3.5 Mean soil respiration rates expressed as CO_2-C ratios in ppm C for both sites at EV Smith in October 2020.
Full Rate N = + Control
Control = -Control/Non-Treated

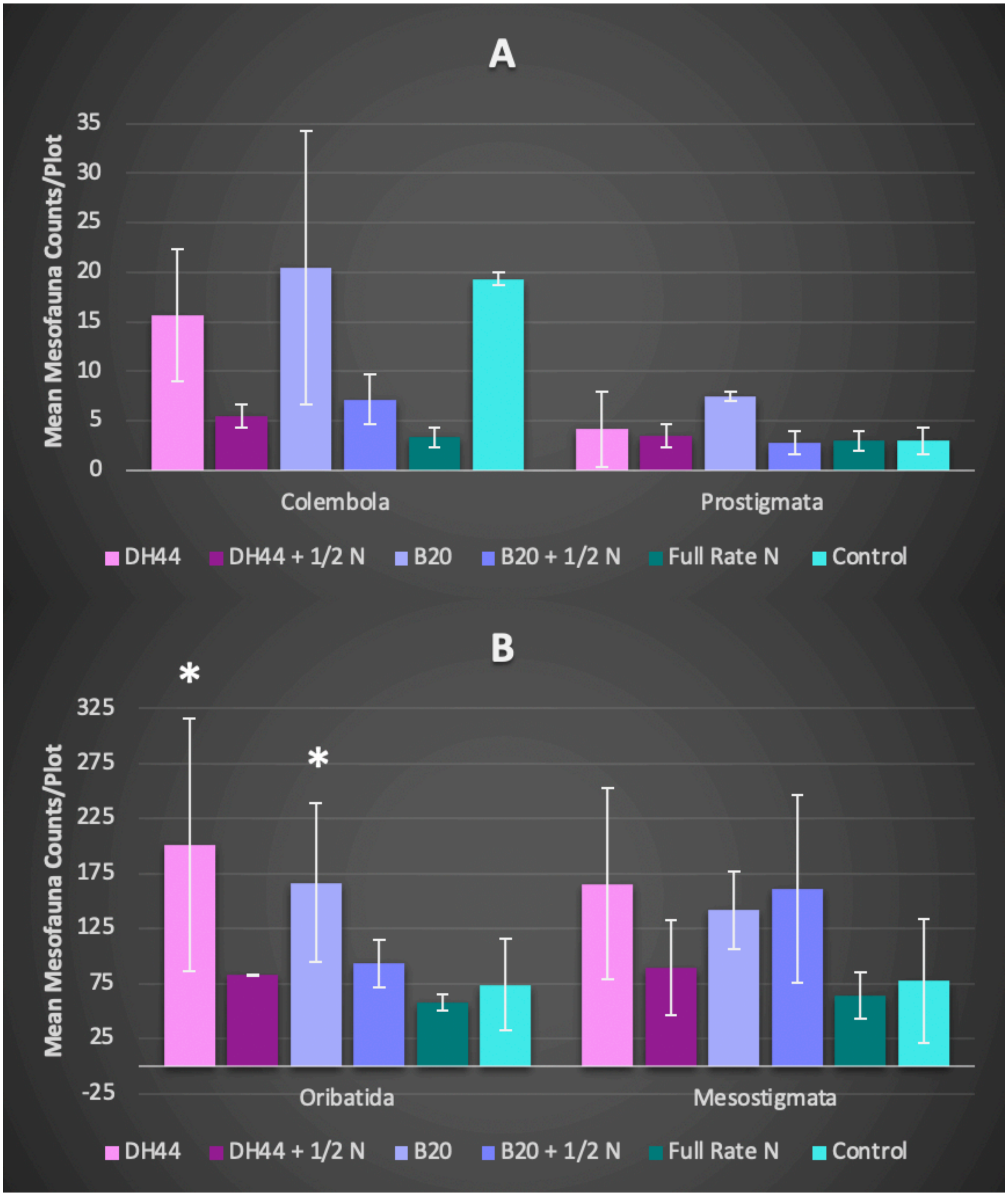


Figure 3.6 Mean mesofauna counts for both sites at EV Smith in October 2020.
A. Mean collembolan and prostigmatid counts per plot
B. Mean oribatid and mesostigmatid counts per plot
Plots were 3m^2 in size and samples were collected with 2 soil cores per plot.
Full Rate N = + Control
Control = -Control/Non-Treated

Conclusion:

The results from these studies suggest that these PGPR strains can be effective as biostimulants in bermudagrass under certain conditions. Previous greenhouse and field studies indicate that both B20 and DH44 are effective at increasing growth promotion in bermudagrass. The current study reinforces these conclusions on a large-scale field study basis. Furthermore, previous studies on stockpiled bermudagrass indicated that, when applied with half of the recommended rate of nitrogen fertilizer, Blend 20 and DH44 can yield similar forage biomass and nutritional value as a full rate of nitrogen fertilizer. The current study reinforces these results in regard to forage biomass production as well. These results indicate that PGPR could be a novel tool in decreasing nitrogen applications in forage production systems. While the results of forage biomass are significant, it is only one component of forage management. Samples are also being analyzed for forage quality and this may provide additional insights into the utility of PGPR for forage production.

While there were no direct influences from PGPR on arthropod populations, there could be benefits from PGPR regarding increased tolerance to damage. Previous studies have shown that these PGPR strains can increase resistance to drought and insect damage from multiple species through enhancing the root system of the bermudagrass. This resistance seems to be increased with more applications over time.

The resulting trends identified when studying the effects of nitrogen fertilizer and PGPR on soil fauna suggest that reduction of nitrogen could also improve soil health. Populations of oribatids were significantly higher in plots treated with PGPR than in plots treated with a full rate of fertilizer and control plots. Which also indicates that applications of PGPR could positively impact soil health directly in addition to decreasing nitrogen applications. While statistical analysis is limited at this time, trends show promising results in increasing soil mesofauna populations and soil respiration. With the lack of research surrounding soil mesofauna, a second year of these results could allow us to better understand the negative effects of nitrogen fertilizers on soil health and benefits yielded by PGPR applications.

As the negative environmental impacts from chemical fertilizers continue to worsen and expand, identification and evaluation of biological alternatives will become more important. In addition to forage production, the PGPR could also be beneficial for turfgrass management. Forage production can be seen as a proxy for low maintenance turfgrass. Therefore, these results could also prove importance in management of low input urban lawns. This is especially important in coastal areas affected by dead zones where fertilizer use can be restricted by municipal governments. Overall, these strains of PGPR show potential as biostimulants in bermudagrass. Future studies are needed to determine long-term PGPR effects and benefits that could lead to the commercialization of these strains.