SUSTAINABLE HIGHWAY TURF FOR FLORIDA AND SOUTHEASTERN UNITED STATES SOILS

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CHAPTER 1

SUSTAINABLE VEGETATION FOR FLORIDA RIGHTS-OF-WAY

Background

The scenery of Florida highways may change depending on the location and season, but one aspect of the roadway system remains consistent. That is, many of Florida's rights-of-way are in vegetative cover, principally grasses, and they provide safety, economic, environmental, as well as aesthetic contributions to the roadway system. Bahiagrass (*Paspalum notatum* Flüggé) fulfills all four of these needs for the Florida peninsula and much of the U.S. Southern Coastal Plain.

According to U.S. Department of Transportation and Federal Highway Administration, Florida's total public road length in 2015 was an estimated 122,700 miles (FHSA, 2015), and roughly 24 acres per mile of easement in bahiagrass. This equates to approximately three million acres of bahiagrass associated with Florida roads. Most of these vegetated or bahiagrass acres, are maintained by the Florida Department of Transportation (FDOT), where in 2014 approximately $33.5 million was allocated to manage vegetative acres, and 25% of that budget was allocated to mowing costs (Harrison, 2014).

Mowing large areas or strips along the highway requires sizeable equipment that can cut a wide swath quickly to reduce overall management costs and to remain within state budget schedules. However, the larger diameter blade length can increase grass scalping hazards over uneven terrain. Additionally, the larger equipment cannot easily and safely accommodate more extreme terrain, such as severe inclines.

Mismanagement is one of the contributing factors to periodic declining bahiagrass swards on rights-of-way. Similar to a lawn, repeated, aggressive mowing that scalps the grass close to the soil surface jeopardizes adequate regrowth and makes the plant more susceptible to pests, diseases and invasive weeds. In addition, the lack of routine fertilizer applications to an already nutrient-poor roadside soil may compromise plant health and result in a greater chance for stand decline. On sloped terrain, this will eventually result in soil erosion. An ideal roadside vegetative cover that requires less frequent mowing with little to no fertilizer applications may greatly improve plant and soil health, as well as benefit rights-of-way management and state appropriated budget outlays.

When grass vegetative cover is compromised, the soil becomes exposed and weeds can establish. Weeds encroaching into a grass sward might appear well-vegetated and green from a distance, but many annual weeds do not typically spread evenly over the ground or form a fibrous root system like a sod-forming grass. The growth habit of many weed species results in spotty vegetative cover. This is more easily observed after mowing, where the main stem that was previously camouflaged by above ground vegetation is all that remains following mowing (Fig. 1-1). This new area of exposed soil is now more susceptible to erosion, particularly during heavy rain events (Nearing et al., 2005; Pimentel et al., 1995).

A soil’s susceptibility to erosion depends on soil texture, and to a lesser extent, soil organic matter (Pimentel et al., 1995). Without adequate root growth and biomass turn-over, soil organic matter (SOM) content remains low. Soils with a greater silt and gravel content and increased slope severity are more prone to water erosion.

Additionally, finer textured soils (greater clay content) reduces water infiltration rates, while increasing SOM improves water infiltration. Bahiagrass is noted for its ability to increase SOM and improve water infiltration, due to its massive, fibrous and deep root system.

Fig. 1-1. I-10 right-of-way on embankment. Bahiagrass decline, followed by erosion and weed infestation exposes increasingly more soil over time.

Maintaining a heathy, sod-forming grass is one of the simplest and most economical ways to keep some of our more erosion-prone highway easements serviceable and attractive. Preserving grass health and function under today's funding constraints and desire to reduce fertilizer inputs, present challenges to state and local road departments. This report investigated options for improving the health and function of this grass-based landscape by including a perennial legume into the system to provide a source of nitrogen (N) fertility and other ecosystem services, as well as reduce risk if one sward species is attacked by pests or disease. The grass species under consideration is bahiagrass (*Paspalum notatum* Flüggé), and the perennial legume is rhizoma peanut (*Arachis glabrata* Benth.).

Bahiagrass

Bahiagrass, (*Paspalum notatum* Flüggé), is a warm-season, perennial grass, native to subtropical regions of South America. It is grown under similar climates world-wide. In the southern United States, bahiagrass is grown primarily as a pasture grass or hay forage, and as turf for public spaces and highway rights-of-way (Gates et al., 2004; Blount and Acuña, 2009). Bahiagrass performs best in moderately acid, sandy soils (Rana et al., 2013). In addition, it prefers well-drained soils, but it can also survive in temporarily saturated/flooded soils (Sigua et al., 2013).

Physiology

In the southeastern United States, the active growing period for bahiagrass is approximately April through September. Bahiagrass is typically propagated by seed, although sod is often used when a quick vegetative cover is required (for example, lawns or easements). Considered an obligate, long-day plant, bahiagrass produces the most seed heads under the longest days. Bahiagrass produces numerous seeds on two racemes (seed heads) atop a long stem. Seed head number, seed count, raceme length, and culm length vary according to cultivar and environmental conditions.

Bahiagrass plants establish quickly if seeded during the rainy season, June through August in Florida (Newman et al., 2010). During the cool-season, bahiagrass enters dormancy but there is some variation in this response. For example, ‘Argentine’ enters a strong dormancy upon the first moderate frost or freeze and will often not break dormancy until the following spring. In comparison, diploid types, such as ‘Pensacola’, will often re-establish vegetative growth if temperatures rise significantly after the frost. During dormancy, bahiagrass sod turns brown and maintains a cover over the soil surface, thereby minimizing the chance for soil erosion if the sod is healthy and thick.

A strong sod cover relies on healthy, aggressive tiller production. The quantity and quality of bahiagrass tillers depend on the amount of energy stored in rhizomes and roots. This stored energy is used for leaf and seed production. It is interesting to note that greater tiller densities were observed after a cutting or defoliation height of 2 cm compared to 12 cm or 17 cm (Hirata, 2000). Similar results in a 2011 study found an increase in tiller growth with a shorter cutting height, 4 cm compared to 8 cm (Interrante et al., 2011). This helps to make bahiagrass a good forage grass under grazing pressure. However, constant removal of all leaves degraded bahiagrass swards by decreasing tiller density, leaf production and rhizome mass (Hirata and Pakiding, 2003). To add to this, during a grazing frequency study in 2009, bahiagrass plots grazed at two weeks had fewer tillers than at four weeks, 842 tillers m^{-2} and 984 tillers m^{-2} respectively (Vendramini et al., 2013). Hirata (2000) also reported that increased N applications resulted in greater tiller density. As expected, a combination of moderate grazing/cutting frequency and adequate N fertilization benefited bahiagrass sod health and production.

Bahiagrass can spread across the landscape via rhizomes. Rhizomes not only increase groundcover, but they provide nutrient and energy (carbon) storage. Bahiagrass stores photosynthates (non-structural carbohydrates) in rhizomes, as well as in stems. This characteristic is often found in tropical or subtropical plants, which can survive occasional subfreezing temperatures (Interrante et al., 2009). The rhizomes are crucial to the plant’s survival during defoliations (frequency and amount), extreme weather conditions, and dormancy, as well as foot or vehicular traffic.

Bahiagrass is known to have a deep and extensive root system compared to other warm-season grasses. Roots, along with rhizomes located near the soil surface help stabilize the soil. A deep root system is essential during periods of drought where soil moisture and some plant nutrients may be available in the subsoil depths. During establishment, bahiagrass roots develop at a constant rate when no physical limitations are present (Acuña et al., 2010). Bahiagrass forms root hairs, but unlike bermudagrass and other warm-season grasses, bahiagrass root hairs account for a smaller percentage of total root length. For example, Argentine bahiagrass root hairs accounted for 32% of root length and 29% for Pensacola bahiagrass, compared to 77% for ‘Tifway’ bermudagrass (Green et al., 1991). The root hairs increased the total root length, in turn increasing absorption surface area for water and nutrient uptake.

Bahiagrass roots are able to extract soil water and some nutrients, such as NO_3-N, from the subsoil, thereby contributing to their success as a great vegetative cover on rights-of-way, where water and nutrients are typically limiting. Beaty et al. (1975) reported that bahiagrass produced a relatively larger root mass without N fertilization, compared to many other perennial grasses, such as bermudagrass. Among six tested cultivars, ‘Argentine’ (tetraploid) produced greater rhizome+root biomass than ‘TifQuik’ and ‘UF-Riata’ (diploids bred for high above-ground yields) in an Orangeburg series soil, under no N fertilization.

Bahiagrass Cultivars and Uses

Bahiagrass cultivars have been bred for use as turf, utility turf, or as forage for livestock. Bahiagrass can be split into two distinct botanical types or ploidy types, diploid and tetraploid. Diploid bahiagrass typically has long, narrow leaves up to 35 cm long, having two to three racemes and a plant height ranging from 40 to 70 cm (Blount and Acuña, 2009). ‘Pensacola’, a diploid bahiagrass cultivar, is most commonly grown for pasture and forage production, due to its moderate cold tolerance and high forage yields. ‘Tifton 9’ is another diploid bahiagrass that produces approximately 30% more forage than Pensacola (Blount and Acuña, 2009), while TifQuick bahiagrass yields somewhat more than Tifton 9 and has more uniform, early germination (Blount and Acuña, 2009; Newman et al., 2010).

The most common tetraploid bahiagrass in the U.S. is ‘Argentine’, which has broad leaves that are flat or folded, and plant height ranging from 15 to 50 cm tall. This bahiagrass is favored for sod use, such as along roadways, and Argentine bahiagrass produces fewer seed heads than Pensacola (Blount and Acuña, 2009) and has dark, broad leaves which may be more desirable for home lawns. Argentine may might be somewhat less suitable for hay production due to its susceptibility to ergot in the seed heads, which is toxic to cattle (Bacon et al., 1986). ‘Riba’ is a low-growing, turf-type bahiagrass that may require less mowing than Argentine and seems less susceptible to ergot infection (Blount and Acuña, 2009). However, there is no commercial seed production in the United States.

Bahiagrass Stand Decline

Frequent, repeated aggressive mowing or scalping can negatively impact a bahiagrass sward to the point that the plant is no longer able to recover. Bahiagrass stand decline can result in greater environmental problems. The lack of plant biomass (above- and below-ground), leaves a soil susceptible to water erosion. Stand decline aggravates erosion problems, especially on slopes and compacted surface soil near waterways. Lack of vegetation over highly leachable soil types may contribute road contaminants to groundwater. Stand decline may result from a lack of essential nutrients in the soil and/or disease pressures. These problems are exacerbated by improper mowing practices.

While bahiagrass has few disease and pest problems, it is still susceptible to a few fungal diseases, and in some cases a portion of or an entire stand may be killed. The humid, warm weather in Florida and low mowing height practices increase disease incidences (Blount and Acuña, 2009; Goodman and Burpee, 1991). For example, Dollar spot disease, *Clarireedia monteithiana*, can destroy the leaves of bahiagrass, which in turn decreases the plant area available for photosynthesis and result in suboptimal plant growth. According to multilocation and multiyear observations, this disease was observed to be less detrimental in tetraploid, Argentine, compared to diploid, Pensacola (Blount and Acuña, 2009). While not exclusive to bahiagrass, dollar spot is also found in other grasses such as bermudagrass, *Cynodon dactylon*, and zoysiagrass, *Zoysia spp*, (Goodman and Burpee, 1991). Root rot diseases, such as *Rhizoctonia solani* (Brown patch) and *Gaeumannomyces graminis* (Take-All) have been routinely identified in bahiagrass fields showing symptoms of decline (C. Mackowiak, personal communication).

Bahiagrass can grow well in low-input systems; however, if soil nutrients are lacking or were depleted through management, erosion or leaching events, then the stand may go into decline. For vigorous plant growth, the optimal pH for bahiagrass was 5.5 in a Smyrna sand, compared to growth at either 4.5 or 6.5 (Rana et al., 2013). Additionally, bahiagrass can remove over 200 kg N ha^{-1} through its above-ground biomass (Mackowiak et al., 2008), under high N fertility, but values decline to about 30 kg N ha^{-1} without N inputs (Santos et al., 2019). However, they also estimated that 8 to 10 kg N ha^{-1} was contributed through biological N_2 fixation (BNF). The amount of BNF associated with bahiagrass stands is not likely adequate to replace N losses due to sward mismanagement over the long-term, but it may partially offset N depletion problems, compared to using other perennial grass species as a monoculture.

Rhizoma Peanut

Physiology

Rhizoma peanut, *Arachis glabrata* Benth., is a warm-season, perennial legume native to several subtropical regions in South America, particularly in areas also associated with bahiagrass. Like bahiagrass, rhizoma peanut can be grown on U.S. Southern Coastal Plain and sandier soils of Peninsular Florida (Sainju et al., 2006; Quesenberry et al., 2010). Once established, it survives under low fertility and minor periods of drought. There are few known pest or disease problems associated with this species. However, rhizoma peanut in the southern U.S. is infected with Peanut Stunt

Virus (PSV) (*Cucumovirus sp*), that has resulted in some yield decline in ‘Florigraze’ (Blount et al., 2002), but the virus appears to express itself in Florigraze more than in other cultivars. Overall, it has not been a major concern to producers. While rhizoma peanut appears similar to the annual peanut, *Arachis hypogaea*, *A. glabrata* is a perennial that produces few seed. Rhizoma peanut cultivars have been developed as ornamental groundcover and as forage. There are genotypic differences in canopy height, biomass, flowering, and shade tolerance (Anderson et al., 2015). Rhizoma peanut tends to accumulate more N in its leaves than stems. For example, rhizoma peanut grown in shade (lower leaf:stem ratio) had less nutritive value than rhizoma peanut grown in full sun (Johnson et al., 2002) and cultivars having greater leaf:stem ratios tended to also have greater crude protein (Mullenix et al., 2016). When mowed, the N tied up in the leaves and stems eventually becomes available to the surrounding plant community.

Rhizoma peanut is established vegetatively from rhizomes that are often planted in February through June, although sometimes later plantings into September have been successful. Complete rhizoma peanut establishment has been reported to take two to three years, on average (Venuto et al., 2000). However, new herbicide registrations since 2000, has lessened establishment time to one season in some cases (Castillo et al., 2013). Like Argentine bahiagrass, rhizoma peanut goes into dormancy after the first fall frost or freeze and it will typically not re-emerge until spring (March in North Florida). Under warmer conditions, rhizoma peanut will remain green year-round, although growth will slow considerably. A monoculture of rhizoma peanut that has entered dormancy often results in some exposed soil, as leaves and stems are shed.

This makes its winter appearance somewhat unattractive and may allow for some soil loss through wind and water erosion during this period.

Rhizoma Peanut Cultivars and Uses

Rhizoma peanut has been grown as a forage in the southeastern U.S. over the past few decades and more recently, some cultivars are being used as ornamental groundcover. Each cultivar has distinct physical and compositional characteristics that determine how and where it will be used. The rhizoma peanut varieties most widely planted as forage have been ‘Florigraze’ and ‘Arbrook’ (Williams et al., 2017), although there are two more commercial cultivars available, ‘Peace’ and ‘Tito’ that are quickly gaining acceptance. Florigraze and Arbrook are more susceptible than Peace and Tito to visual symptoms of Peanut Stunt Virus (Quesenberry et al., 2010). Florigraze can produce between 10-12 Mg ha^{-1} of dry matter per year in Florida (Castillo et al., 2013), which is comparable to alfalfa in terms of crude protein content and digestibility (Castillo et al., 2013; Terrill et al., 1996). Foster et al. (2011) found that ensiled rhizoma peanut had 15.8% crude protein (dry matter basis), compared to bahiagrass at 10.9%. Crude protein content was found not to be greatly impacted by grazing, but it does vary somewhat by cultivar (Mullenix et al., 2016).

‘Ecoturf’ and ‘Arblick’ are two cultivars most commonly planted in urban medians as ornamentals, due to prolific flowering and canopy height remaining low during the growing season (Prine et al., 2010). However, Ecoturf establishes more quickly than Arblick (Prine et al., 2010). More recently, Ecoturf and ‘Cowboy’ (another prolific flowering cultivar) have been planted as monocultures into Florida urban and highway medians, as attractive, low-input, vegetative cover.

CHAPTER 2

THE ROLE OF BIOLOGICAL N_2 FIXATION IN A SUSTAINABLE LANDSCAPE

Nitrogen Cycle

Dinitrogen gas makes up over 78% of the Earth's atmosphere and approximately 3.9×10^{15} metric tons of N, although there may be over 40 times more global N tied up in bedrock and other parts of the Earth's crust (Havlin et al., 2014). Compared to the atmosphere, oceans hold approximately 2.4×10^{13} metric tons and soils a mere 4.5×10^{11} metric tons (Havlin et al., 2014). Even though there is a vast abundance of atmospheric N_2, most plants lack the ability to convert N_2 to reactive N, such as plant available N_4-N and NO_3-N.

There is a large capacity within the soil ecosystem to store and produce plant available N through different biological and chemical transformation processes. Much of the soil N comes from decomposed plants and animals converted into organic N forms. However, there are many weathered or poorly managed soils where most of the organic matter has been eroded away (wind or water) or mineralized into inorganic N forms that are also more susceptible to run-off and leaching losses. If N fertilizer is not added to the soil, many crops cannot meet their growth potential (N-limited). With an abundance of atmosphere N_2, a plant capable of converting N_2 through BNF has an advantage in an N-limited landscape.

The N cycle (Fig. 2-1) involves fluxes of organic and inorganic forms of N that transfer between soil, bacteria, plant and atmosphere. Dead plant and animal material, or organic matter, is converted to NH_4^+ through ammonification by decomposers (Havlin et al., 2014). Decomposers, such as fungi and bacteria (Stein and Klotz, 2016), are essential for breaking down the organic N into plant-available inorganic N. Ammonia-oxidizing bacteria converts NH_4-N to NO_3-N via the nitrification process. More specifically, NH_4^+ is converted to NO_2^- by nitrosomonas bacterium, and then NO_2^- to NO_3^- by nitrobacter bacterium (Stein and Klotz, 2016). Both NH_4^+ and NO_3^- forms are taken up by the plant and this N can also be immobilized back into organic forms through microbial assimilation (Havlin et al., 2014). Nitrogen can leave the soil through 1) uptake by the plant, 2) conversion to gaseous compounds NH_3 via ammonification, or N_2O and N_2 volatilization by denitrifying bacteria through the process of denitrification (Stein and Klotz, 2016), and 3) leached as NO_3-N through the soil profile or as run-off into groundwater or surface waters.

Release of NH_3, via ammonification, becomes more favorable, with increasing soil pH in the bulk soil (Brady and Weil, 2002) or microsites where conversions are taking place. Nitrous oxide (N_2O) and nitric oxide (NO) are intermediates of denitrification. Whereas N_2O is an important greenhouse gas, NO may contribute to ozone depletion and acid rain formation, via nitric acid (Stein and Klotz, 2016; Brady and Weil, 2002). Nitrogen can also be leached as NO_3-N. Excessive rainfall or a poorly managed landscape leaches NO_3-N through the soil profile due to its negative charge or as run-off into surface waters, resulting in groundwater pollution and eutrophication in water bodies. These consequences are especially detrimental when mineral N fertilizer is added in excess or is poorly managed in the environment. This is a growing concern. For example, according to one bibliometric study, publications addressing nitrate leaching increased by an average rate of 56% per decade from 1960-2017 (Padilla et al., 2018). Additionally, historical N fertilizer use in the US rose quickly (from <0.3 to 9.8 Tg N yr^{-1}) from post WWII, through 2008, although the rate of increase (from 9.8 to 11.4 Tg N yr^{-1}) has slowed since 2008 (Cao et al., 2018). Utilizing more plants capable of BNF, such as legumes, could further reduce the need for soluble, mineral N fertilizer inputs (Reckling et al., 2016).

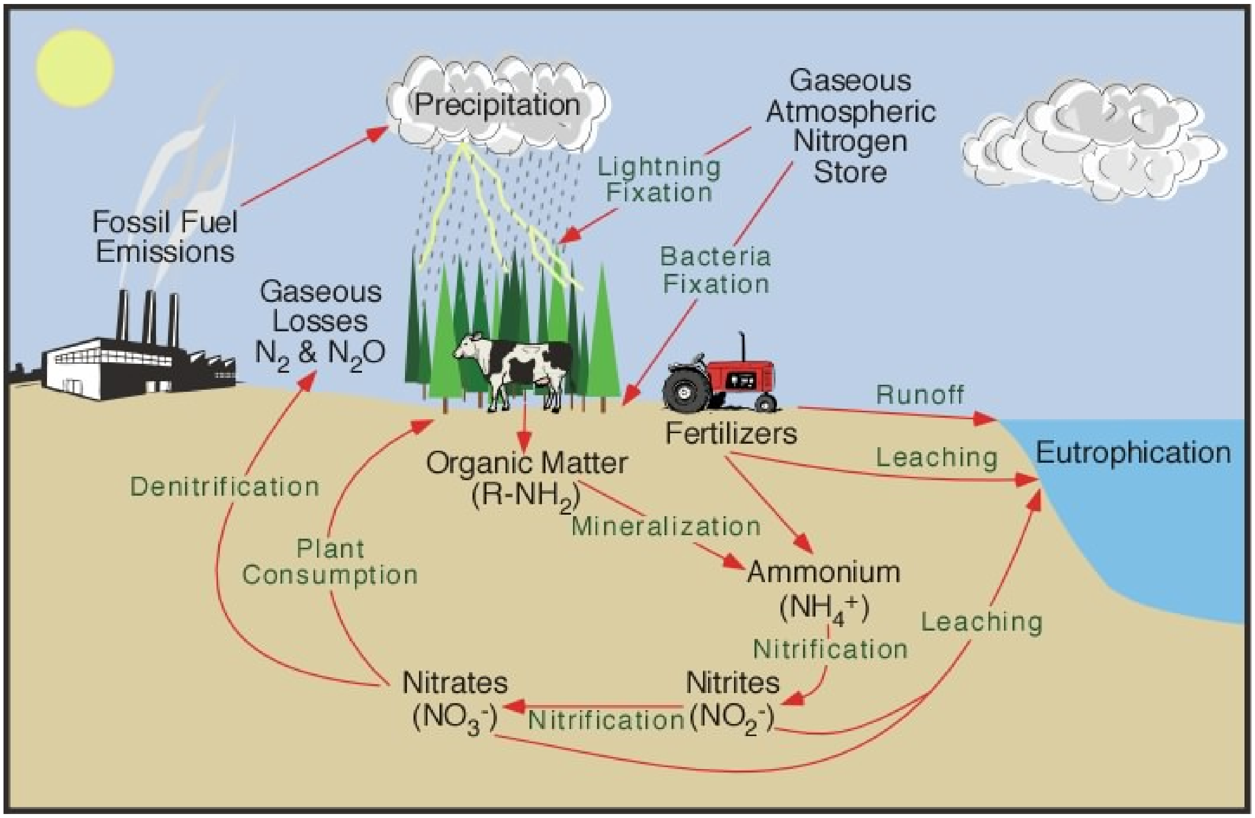

Fig. 2-1. Example of a simplified Nitrogen Cycle (Pidwirny, 2006).

Biological Dinitrogen Fixation (BNF)

Biological dinitrogen fixation can be with free living bacteria or through symbiosis with soil bacteria, collectively called rhizobia. Bacteria that fix N_2 are classified by the means of where their energy derives: 1) heterotrophs rely on other organisms for synthesis and 2) autotrophs can produce energy from chemical reactions or light (Unkovich et al., 2008). Both classes are made up of free-living and symbiotic bacteria species. While most free-living BNF bacteria reside in the soil, some live in aquatic environments such as some cyanobacteria that enhance rice N nutrition grown in paddy soils (Havlin et al., 2014). Depending on soil conditions, heterotrophs can fix approximately 5 to 20 kg N ha $^{-1}$ per year, while autotrophs can fix 20 to 30 kg N ha $^{-1}$ per year (Brady and Weil, 2002; Unkovich et al., 2008). Another free-living bacteria is associative, *Azotobacter paspali*, which was found to be permanently established in the rhizosphere of four tetraploid cultivars of *Paspalum notatum* (Hamdi, 1982). Free-living, non-symbiotic BNF bacteria may improve N nutrition in some grass species like *Paspalum spp*. and further studies are needed to better understand these relationships.

The most commonly known symbiotic BNF associations are with legumes and some other angiosperms (Brady and Weil, 2002). In the case of rhizoma peanut, the development of root nodules containing the *Bradyrhizobium spp* bacteria (Elsayed et at., 2017) allows rhizoma peanut plant to fix atmospheric N_2 in lieu of relying upon soil N fertility (Venuto et al., 2000). Rhizoma peanut is described as promiscuous due to its ability to form associations with different rhizobia species. However, BNF comes at a cost to the host plant, where energy is supplied to the bacteria in the form of carbon. It is estimated that 2 to 3 mg C per mg of N_2 fixed in legume plants (Valentine et al., 2011).

The process of BNF begins with signaling and response between host plant and bacteria. The host plant’s roots release flavonoids into the soil surrounding the root, or rhizosphere that attract the rhizobia to the root (Salisbury and Ross, 1992). These flavonoids activate nodulation genes within the rhizobium (Wasson et al., 2006). Leghemoglobin protein appears pink or red in the nodule, and it is produced to buffer O_2 concentrations in the nodules. Nitrogenase enzyme activity is greatest under a low O_2 environment (Salisbury and Ross, 1992). For this association to be symbiotic, the host plant provides energy in the form of carbohydrates to the bacteriods, and the bacteriods provide the plant NH_4^+ that was reduced from N_2 by nitrogenase enzymes.

The amount of BNF can vary among different leguminous species, and the various soil factors. For example, rhizobia tend to grow in a pH range of 5.5 to 7.5 (Hamdi, 1982) with *Bradyrhizobium* associations tolerating more acidic conditions than *Rhizobium* (Brady and Weil, 2002). Soil pH will affect the presence or absence of certain essential nutrients or minerals. A lower soil pH can drive reactions, resulting in greater nutrient availability for plants and the rhizobia. However, a soil pH that is too low can solubilize some minerals, such as Al or Mn. Aluminum toxicity was found to weaken the interaction between the plant and rhizobia in lower pH soils (Artigas-Ramírez et al., 2018).

As with the animal kingdom, Fe, B and Cu are found to be essential to rhizobia and nodule development (Hamdi, 1982). Although required in small amounts, Mo is a major component of nitrogenase (Havlin et al., 2014; Khan et al., 2014), an enzyme required to help break N_2 bonds. High levels of soil NO_3-N can reduce nitrogenase activity, thereby, inhibiting BNF (Havlin et al., 2014). Plants will bypass energy intensive BNF, in exchange for soil inorganic N, which will eventually decrease rhizobia populations within a legume stand.

The amount of organic matter can either facilitate or hinder BNF. Organic matter retains carbon, nutrients and moisture in the soil. If there is little to no organic matter in highly weathered, sandy soils, nutrients may become scarce and BNF may decrease, along with a decrease of plant health. Organic matter also increases soil buffering from extremely acidic or basic conditions. Under acid stress, the presence of soil organic matter may also buffer against certain metals in the soil, such as aluminum, thereby protecting the plant and rhizobia from Al toxicity (Lawson et al., 1995).

During cold dormancy, below-ground production does not cease. Rhizobia can develop and mature in a temperature range of 0-50˚C but a temperature of 20-28˚C was found to be optimal (Hamdi, 1982). In one study, the majority of the rhizobium strains from cowpea collected from the Sahel Savannah grew at 40˚C (Zahran, 1999).

Lack of soil moisture affects BNF. When compared to shoot and root metabolism, rhizobia nodulation and fixation exhibited greater sensitivities to water stress (Zahran, 1999). Soybean nodule numbers were shown to decrease with decreasing soil moisture, along with nodule size under extreme drought (Zahran, 1999). Water is required for nutrient transport of ions to the plant roots. Without nutrients, BNF decreases. Soil texture controls soil water holding capacity. Soils with a higher percentage of clay, such as Ultisols, have larger water holding capacities. Rhizobia have been found to have an increased number of root nodules in heavy textured soils, or soils with greater clay content (Al-Saedi et al., 2016). However, if the soils are too heavy or stay too saturated, rhizoma peanut may not perform as well as in more coarse soil types. Most soil types in Florida contain over 85% sand and therefore are attractive for rhizoma peanut production.

The Fate of Biological N_2 Fixed N in Soil

The potential amount of BNF among rhizoma peanut cultivars ranges from 120 to 210 kg N ha^{-1} per year (Dubeux et al., 2017) and is similar to legumes such as alfalfa with a range of 50 to 300 lbs N acre^{-1} per year (Havlin et al., 2014). Neighboring soil organisms and plants can indirectly benefit from BNF legumes. As the host plant grows or during winter dormancy, leaves, roots and root nodules rich in N die or slough off and become newly added organic matter to the soil. Soil decomposers utilize the material and smaller N organic compounds are utilized by microorganisms and assimilated into microbial and fungal biomass. Eventually inorganic N is released and made available for neighboring plants, via mineralization. While this method of N transfer may be considered slow due to decomposition, etc., crop rotation systems that include legumes will often benefit from such a process (Reckling et al., 2016; Li et al., 2018).

The roots of the rhizobia's host plant can also transfer N through root exudates and it is found to be the more predominant mode of N transfer in younger leguminous species (Thilakarathna et al., 2016). Structure of a neighboring plant roots is important when optimizing N recovery from a legume, and when in competition with other plants. The depth and area of the host's roots may become more important when soil moisture is less, since drier soil zones keep the N exudates immobile. Plants with fibrous roots, such as bahiagrass, compared to plants with one main taproot have been found to be more effective at capturing soil N that originated from the host's roots (Thilakarathna et al., 2016).

Methods for Assessing Biological Dinitrogen Fixation in Soil

Measuring the amount of N fixed in a system has proven to be difficult. Nitrogen transformations by microorganisms are occurring in the soil all the time regardless of the presence or absence of symbiotic BNF. The origination of the N isolated may not be known when taking simple N measurements. Different methods of assessing BNF in the soil brings challenges and some advantages and disadvantages.

The Acetylene Assay method (Unkovich et al., 2008) is used to detect nitrogenase in a system which is the enzyme responsible for fixing N. Acetylene gas, in the presence on nitrogenase, is reduced to ethylene. Gas chromatography is used to measure ethylene, thus measuring nitrogenase activity. While this method is quick and does not require advanced technological machines, it only can measure the relative rates of nitrogenase activity and not the actual quantities (Unkovich et al., 2008) and samples to be measured are time limited. Another disadvantage is measuring a specific plant or section of root that is actively growing in the field with other plants and microorganisms. Since there are other free-living BNF bacteria in the soil, any unwanted soil particles not removed would be accounted for in the measurement and would be in error. This problem also has a chemical disadvantage as the shaking of the roots results in an increase in the oxygen diffusion resistance on the nodule (Minchin et al., 1986) and resulting in a decrease in nitrogenase activity.

Another method for measuring BNF is by ^{15}N stable isotope. This method uses a mass spectrometer to measure, through combustion, the differences in plant isotopic composition of N_2 and plant-available soil N (Ledgard et al., 1985). Nitrogen has two stable isotopes, ^{15}N and ^{14}N, with the latter being more abundant. In the atmosphere the two isotopes, $^{15}N/^{14}N$, remain constant, allowing atmospheric N_2 to be used as the standard for mass spectrometer analyses (He et al., 2009). Unlike the acetylene assay method, BNF through this method can be quantified and used in mixed plant species, (fixing and non-fixing), growing together in a field. Some challenges of this method are the expense of equipment and the preparation of the sample to analyze.

There are two different ways to use the stable isotope method: 1) natural abundance and 2) dilution or labeling. For natural abundance, the N measured in the BNF plant represents both, N_2 fixed from the atmosphere plus plant-available, soil mineral N. For this to work, the isotopic signatures of the atmosphere and soil need to be different enough to track source. Both applications require a reference plant that does not fix N_2 and grown in the same medium, without any interaction with the BNF plant (Ledgard et al., 1985). With natural abundance, the reference plant is used to approximate the available soil N that the BNF plant may can take up (Peoples et al., 2015). The challenge of this is to select a reference plant that has similar growth characteristics as the BNF species. Ideally, a non-BNF mutant of the BNF species is ideal but for many legume species, it does not exist. The natural abundance method can be used in a field where N_2 fixers and non-fixers reside in proximity. While this is an advantage, only measuring plant shoots instead of the whole plant results in isotopic fractionation (shoot $\delta^{15}N$ values trend lower than atmospheric N_2 and other plant parts). This is addressed by measuring $\delta^{15}N$ taken from the BNF legume grown under no soil N or B-value (Unkovich et al., 2008; Peoples et al., 2015). The B-value is subtracted from the $\delta^{15}N$ of the reference plant.

The ^{15}N dilution or labeling application follows the same principles as the natural abundance method in terms of reference plant and BNF plant, but an equal amount of ^{15}N labeled fertilizer is applied to the surface of the soil of both plants. By spiking the soil, there is no need to account for the isotopic fractionation (B value) because the amount of ^{15}N label is much greater than the natural variation in ^{15}N (Peoples et al., 2015). Because the reference plant is acting as the value of the soil N for the BNF plant, soil conditions (water and temperature) should be kept similar among the plants.

Assessment of ^{15}N Natural Abundance and Dilution Techniques on Planted Bahiagrass and Rhizoma Peanut

The ^{15}N natural abundance method for assessing BNF requires no plant manipulation until it is time to harvest the tissue of interest. However, there are potential limitations to its usefulness. It is difficult to account for all the factors affecting ^{15}N:^{14}N ratios in plants, which include soil, environmental, BNF community composition and abundance, and mycorrhizal influences, to name a few (Craine et al., 2015). Legumes using BNF will often have $\delta^{15}N$ signatures similar to atmosphere values ($\delta^{15}N = 0$), and they are typically less enriched than plants that do not rely on BNF. However, if the soil δ15N signature is not significantly different, it becomes more difficult to distinguish between plants with BNF and those without (Unkovich and Pate, 2001). In those situations, ^{15}N enrichment might be an option.

Rhizoma peanut and bahiagrass were grown in soils from two Florida locations in the greenhouse in order to determine if either 15N natural abundance or 15N dilution techniques were applicable in assessing symbiotic BNF in rhizoma peanut and possible associative BNF in bahiagrass.

Materials and Methods

Experimental design and columns

The experiment was designed to collect soil and plant plugs (rhizoma peanut and bahiagrass) from two different plot locations having different soil types for assessing potential BNF based upon tissue natural abundance ^{15}N and ^{15}N dilution techniques. Since there are reports of potential associative BNF with *P. notatum*, pearlmillet *Pennisetum glaucum* L. 'Tif-leaf' was also included as a species without BNF (Lee et al., 1994). The experiment was a randomized complete block design and 2 x 6 factorial (2 soil types, 6 plant types) with 3 replications. Two cultivars of rhizoma peanut, *Arachis glabrata* Benth, Ecoturf and experimental 'Quincy 6B' rhizoma peanut and two cultivars of bahiagrass, *Paspalum notatum* Flüggé, Argentine and experimental 'Dwarf F9' were tested as monoculture plantings in the same soils where they originated. Millet seed was planted in both soil types.

Plastic (PVC) columns, 60 cm height and 15 cm diameter were used. Each contained a PVC cap and drain valve at the bottom of the column for controlling drainage. The bottom 30 cm of the column was filled with rinsed play sand to maintain good drainage and to reduce the amount of test soil required to fill each column. The soil for each column was packed into a 15-cm diameter x 30 cm deep plastic liner that fitted the inside of the column, on top of the sand layer. Slits were cut through the bottom of the liner to allow for drainage.

Soil and plant preparation

Soil from each location (0 to 30 cm depth) was collected, air-dried, and passed through a 2 mm sieve. North Florida Research and Education Center (NFREC) (30.5496, -84.6001) soil was characterized as Loamy, kaolinitic, thermic, Arenic Plinthic kandiudult, Fuquay series (Soil Survey Staff, 2016). Gulf Coast Research and Education Center (GCREC) (27.7610, -82.2236) soil was characterized as Sandy, siliceous, hyperthermic Oxyaquic Alorthod, Zolfo series (Soil Survey Staff, 2016). Soil characteristics are given in Table 2-1.

Table 2-1. Mehlich 3 extractable nutrients from soils sampled at two Florida locations.

Parameter	NFREC	GCREC
pH	6.1	5.2
	-----------mg kg^{-1}------------	
Total Kjeldahl N	247	242
P	39	113
K	109	8
Mg	55	10
Ca	284	130
S	7	37
B	0.7	1.2
Zn	6.4	1.9
Mn	98	1.7
Fe	171	34
Cu	1.5	2.9

Argentine and experimental Dwarf F-9 bahiagrass and Ecoturf and experimental Quincy 6B rhizoma peanut plant plugs (15 cm diameter x 10 cm deep) were removed, with a golf hole cutting device from field plots at both locations. Each plant was planted in its corresponding soil of origin. Millet was sown at 10 seeds per column. After the millet plants reached approximately five to six centimeters in height, extra plants were removed, leaving five plants of roughly equal size per column. After the initial transplant, deionized water was used to water columns during the study period. Any emerging weeds were removed with tweezers to not disturb the soil in the columns. No additional nutrients were applied to the soils to better mimic field growing conditions under Florida Department of Transportation (FDOT) management.

^{15}N natural abundance sampling

Above-ground biomass was harvested 27 April 2015 and again, 28 July 2015, prior to spiking with ^{15}N. Only the 28 July 2015 biomass was tested for natural abundance ^{15}N. In each case, rhizoma peanut and bahiagrass treatments were clipped to a 7.6 cm stubble height. The millet was clipped just above the first visible node, leaving a somewhat taller stubble height. Plant samples were dried at 50^{o}C. After drying, the samples were weighed for dry mass and tissue milled to pass through a 2 mm sieve. Dried samples were stored in plastic bags, in the dark, under ambient (laboratory) temperature conditions. The prepared samples were ball milled, using a Retsch Mixer Mill MM400 (Verder Scientific, Haan, Germany) at 25 Hz for 9 min. Samples were then analyzed for total N using a CHNS analyzer through the Dumas dry combustion method using an Elementar Vario Micro Cube (Elementar Americas Inc., Ronkonkoma, NY) coupled to an IsoPrime 100 isotope ratio mass spectrometer (Isoprime Ltd., Cheadle, UK). The proportion of plant N derived from the atmosphere (%Ndfa) was estimated using the equation described by Shearer and Kohl (1986):

$$\%Ndfa = \left[\frac{\delta^{15}N_{reference} - \delta^{15}N_{N2-fixing\ legume}}{\delta^{15}N_{reference} - B}\right] * 100$$

Where $\delta^{15}N_{reference}$ is the $\delta^{15}N$ value for the non-BNF millet, $\delta^{15}N_{N2\text{-fixing legume}}$ is the $\delta^{15}N$ value for the symbiotic BNF rhizoma peanut and presumed, associative BNF bahiagrass in this study, and *B* is the value determined when growing Ecoturf (*B* = $^{-}$0.46), Quincy 6B (*B* = $^{-}$0.01) Argentine (*B* = $^{-}$1.54), and Dwarf F-9 (B = $^{-}$0.46), in N-free, sand culture at NFREC-Quincy, 2015.

^{15}N dilution test

On 30 July 2015, 10 mL of a tracer ammonium Ammonium-$^{15}N_2$ sulfate (10 atom% excess ^{15}N) (Sigma-Aldrich, St. Louis, MO) solution was added to each column, carefully avoiding plant tissue. Following application, 200mL of deionized water was applied to move the tracer beneath the soil surface. On 06 September 2015 through 13 September 2015, deionized water was added to each column to collect 1.5 pore volume of leachate. The final harvest was performed on 14 September 2015. The aboveground biomass from each column was removed at the soil surface and weighed for fresh mass. The plastic liner was then slipped out of each column in order to sample soil and roots. A few roots had grown through the slits in the plastic liner and into the sand in the lower half of the column. The roots within the liner were rinsed with deionized water to remove adhered soil and dried at 50^{o}C for at least one week. After drying, the above- and below-ground tissue samples were weighed for dry mass and processed, as described under the natural abundance section for ^{15}N analysis. The proportion of plant N derived from the atmosphere (%Ndfa) was estimated using the equation described by Boddey et al. (1983):

$$\%Ndfa = \left[1 - \frac{\delta^{15}N_{N2-fixing\ legume}}{\delta^{15}N_{reference}}\right] * 100$$

Data was analyzed using Proc Mixed from SAS 9.4 (SAS Inst., 2009). Fixed effect was Treatment. Block and interaction of block x treatment were considered random effects. The LSMEANS were compared using the PDIFF procedure adjusted for Tukey's test. Differences were significant at $P \leq 0.05$.

Results and Discussion

NFREC soil natural abundance $\delta^{15}N$ was 1.75 ±0.71 prior to use. The GCREC soil was not measured but it is reasonable to expect that values were similar, based on planted column results. This will be verified at a later date.

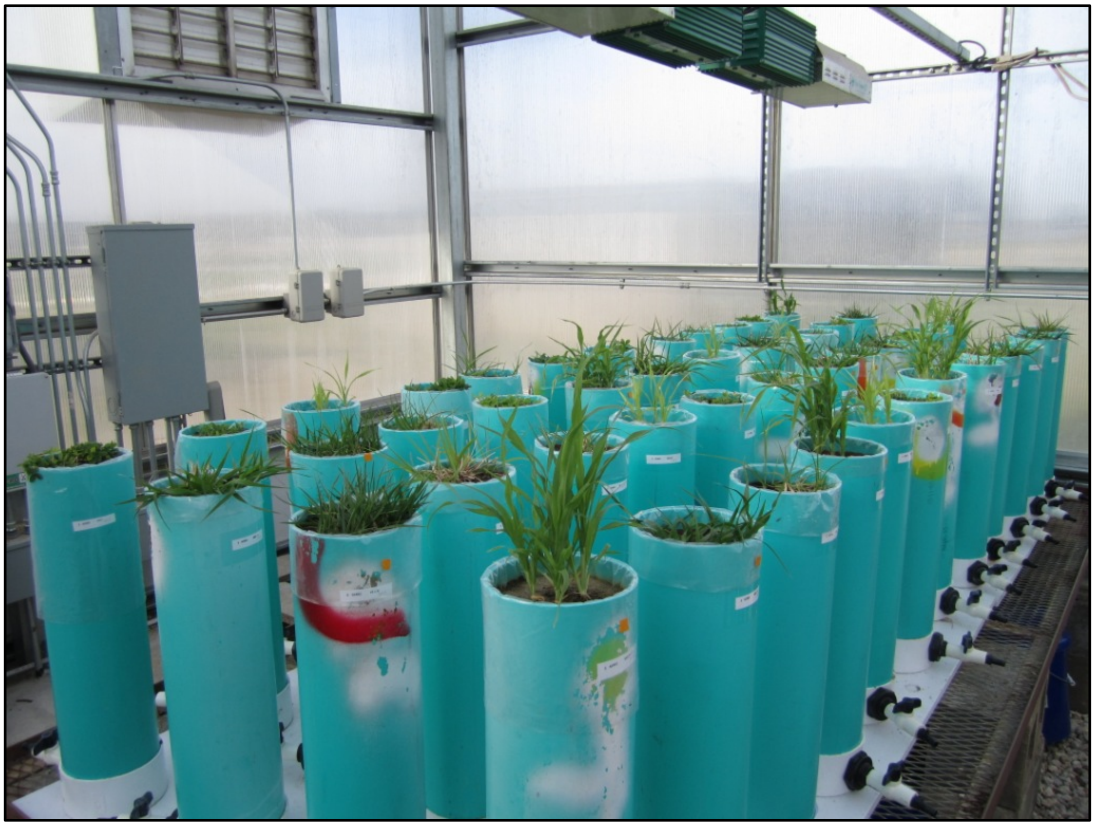

Fig. 2-2. Planted columns on 05 Aug 2015. This study was part of a larger test that included grasses receiving additional N fertilization.

The lightly fertilized millet was used as a reference plant for both, the bahiagrass and rhizoma peanut. The natural abundance method was tested on plant shoot tissue clippings taken in July. Shoot tissue δ^{15}N(‰) were below 1 in all cases (Tables 2-2 and 2-3), with the Ultisol soil expressing all negative values. Natural abundance %Ndfa ranged from -89 to over 200% of total N uptake under Ultisols, and yet they were not significantly different, thereby the data demonstrating large variability. In comparison, the values for GCREC were somewhat closer to what was expected; low %Ndfa for the grasses and high for the rhizoma peanut cultivars (Table 2-3). Although natural abundance is an attractive option for testing many plant systems for BNF, it seemed to result in inconsistencies and questionable results in our system, especially with the Ultisol soil from NFREC (Tables 2-2 and 2-3).

Table 2-2. ^{15}N isotopic tracking of potential BNF of test plants grown in an Ultisol soil.

Forage	Natural abundance		Tracer (tops)		Tracer (roots)	
	δ^{15}N(%o)	%Ndfa	δ^{15}N(%o)	%Ndfa	δ^{15}N(%o)	%Ndfa
Argentine	-0.45	25 *b*	1615 *a*	21 *b*	911 *a*	37 *b*
Dwarf F-9	-0.24	40 *b*	1509 *a*	26 *b*	864 *a*	40 *b*
Ecoturf	-0.65	282 *a*	545 *b*	73 *a*	139 *b*	90 *a*
Quincy 6B	-0.66	-89 *b*	663 *b*	67 *a*	160 *b*	89 *a*
Significance	*ns*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*

In comparison, the tracer (dilution method) provided data with much lower variability and results more consistent within a plant species. Unfortunately, the enrichment appears up to 2 times greater than expected. It is unclear where the error originated. Similar concentrations of ^{15}N applied to field microplots resulted in high shoot values, as great as 50 to 80 $\delta^{15}N$(‰) (Cookson et al., 1990). Regardless, total tissue N was excessively low, at 0.48 ± 0.08 %N for NFREC grasses pre ^{15}N application and 0.53 ± 0.09 %N at final harvest. The GCREC grasses were similarly low, at 0.45 ± 0.03 %N pre and 0.55 ± 0.03 %N at final harvest. This equates to roughly 3% crude protein. It is unclear why plant tissue N was excessively low, particularly since they demonstrated good color and reasonable growth. In comparison, the rhizoma peanut had values of 2.05 to 2.33 ± 0.22 % N across soil types and cultivars.

Table 2-3. ^{15}N isotopic tracking of potential BNF of test plants grown in a Spodosol soil.

Forage	**Natural abundance**		**Tracer (shoot)**		**Tracer (root)**	
	$\delta^{15}N$(‰)	**%Ndfa**	**$\delta^{15}N$(‰)**	**%Ndfa**	**$\delta^{15}N$(‰)**	**%Ndfa**
Argentine	0.23 *b*	3 *c*	1652 *a*	29 *b*	953 *a*	51 *b*
Dwarf F-9	0.85 *a*	9 *c*	1741 *a*	25 *b*	1081 *a*	44 *b*
Ecoturf	-0.68 *c*	118 *b*	736 *b*	68 *a*	217 *b*	89 *a*
Quincy 6B	-0.79 *c*	197 *a*	680 *b*	71 *a*	220 *b*	89 *a*
Significance	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$

Although $\delta^{15}N$(‰) reported high, it resulted in a large difference in values between bahiagrass versus rhizoma peanut (Tables 2-2 and 2-3). These results suggest that rhizoma peanut in this study gained approximately 70% of its N from BNF. This is consistent with what others in Florida have reported from field sites. For example, Dubeux et al. (2017) reported that several rhizoma peanut cultivars in the field had %Ndfa of over 60%, with Ecoturf ranging between approximately 75 to 90 %Ndfa, depending upon harvest month and year. In comparison, Santos et al. (2018) reported field Quincy 6B and Ecoturf values of approximately 60 to 65 %Ndfa. It is interesting to note that when using the millet as a reference plant, we found both bahiagrass cultivars relied on roughly 25% of their N to be derived from associative BNF (Tables 2-2 and 2-3). Boddey et al. (1983) reported low BNF associated with *P. notatum* ‘Batatais’ (%Ndfa of 8 to 25%) when grown in concrete containers outdoors. However, they found no such response with ‘Pensacola’. Root tissue %Ndfa was also calculated in our study, and the relative response was similar to the shoots but %Ndfa shifted higher, suggesting even more activity associated with the roots and perhaps rhizomes. Both, bahiagrass and rhizoma peanut have prominent rhizomes. Microorganisms (bacteria and fungi) have been found associated with rhizoma peanut and bahiagrass that may contribute to N nutrient cycling (Beule et al., 2019). Further investigations into these relationships may help improve sustainable grass-based systems.

CHAPTER 3

INCORPORATING LEGUMES INTO GRASSLANDS

Implications for Wildlife

Soil N from BNF can support neighboring grasses lacking the capacity for BNF (Louarn et al., 2015; Trannin et al., 2000; Vinther and Jensen, 2000). In general, this increases stand nutritive quality over grasses alone, making it more attractive to wildlife, such as deer. Through foraging, urine and dung excretions recycle N and other nutrients back into the system. The nutrient recycling might benefit both, legumes and grasses. In grassland pastures, an increase frequency of application of cattle urine increased the bahiagrass dry matter from 3,040 kg ha^{-1} with no urine application to 4,820 kg ha^{-1} of three applications one year (White-Leech et al., 2013). However, attracting deer or other large wildlife too near roadways is potentially dangerous to both, animal and driver. Knowing the potential for increased wildlife/vehicle traffic interactions, it has not deterred several Florida cities, including Orlando and Tallahassee from increasing rhizoma peanut monoculture plantings into city medians. Florida Department of Transportation has also installed some center median plantings along major highways, but there have not yet been large-scale plantings of rhizoma peanut into bahiagrass.

A north Florida pasture study containing legume/grass mixes, including rhizoma peanut, were shown to attract at least 13 different bee species (Dubeux et al., 2018). Rhizoma peanut is suspected of attracting pollinators, although it rarely produces seed.

One theory of low seed production is due to temperature. As ambient temperatures increased, pollen germination tended to decline (Niles and Quesenberry, 1992).

Implications for Pests and Diseases

Diseases and pests are attracted to legumes as monocultures or in grasses, and the effects may be additive. For example, potato leafhopper infesting alfalfa plants can stunt growth and make the plant more susceptible to other disease pressures, such as Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *Medicaginis* (Faris et al., 1981; Ariss et al., 2007). Alfalfa that showed resistance to potato leafhopper feeding and Fusarium wilt had a 66-85% higher plant survival rate (Ariss et al., 2007). Legumes under attack often reduces BNF production and therefore, N contributions to the sward. Rhizoma peanut is noted for low susceptibility to most pests and diseases. However, it is susceptible to a peanut stunt virus (PSV), genus *Cucumo*virus (Blount et al., 2002). The most commonly grown cultivars are typically infected but often do not display symptoms. Being a stunting virus, there is concern that infection might reduce yields in hay or grazing systems. Florigraze tends to display mottling and malformed leaves under more challenging environments (Baker et al., 1999). Although Florigraze is compatible with bahiagrass, PSV-related symptoms may be a factor in limiting its growth, thereby making Florigraze a less likely choice for rights-of-way plantings.

Longevity of Legume/grass Mixtures

The longevity of individual plant species within a mixed sward depends upon competition for light, water and nutrients. Legumes have been shown to increase soil C and N pools, as is the case with rhizoma peanut (Sainju et al., 2006). The grass component of the sward might benefit from the rhizoma peanut soil N pool. However, if the grass outcompetes the legume, then as the legume component recedes, the grass component will have to rely on other sources of soil N.

Plants that are highly competitive for water or nutrients, within similar spatial regions of the soil profile, may disadvantage one plant species over another. For example, there was greater competition for water and N between citrus and bermudagrass than between citrus and rhizoma peanut (Linares et al., 2010). However, there also may be cultivar options within a plant species that might make a multi-species planting more compatible.

Legume-grass systems have differed in their compatibility and longevity. In a three-year study, the percent of alfalfa dry matter from a mixed alfalfa-bermudagrass sward increased from 20.8% to 66.3%, averaging 50% (Cinar and Hatipoglu. 2014). In comparison, the percent of alfalfa in Rhodes grass (*Chloris gayana* Kunth.) increased drastically in the first year, then decreased slightly in the third year, resulting in an average of 34.3% alfalfa over three years. While alfalfa demonstrates an increase in species composition in perennial grass during the initial establishment year, the likelihood of it remaining stable over the longer term is less likely (Springer et al., 2007).

The amount of N fixed in white clover/grass mixtures has been estimated as 50 to 680 kg N ha^{-1} per year (Wu and McGechan, 2001). Establishment time, species cultivar, and soil characteristics are likely factors in the large range in BNF. Jakubowski et al., (2017) reported red clover planted into a warm-season grass increased sward biomass yields to an equivalency of 112 kg N ha^{-1} fertilization. Tessema and Baars

(2006) established white clover into two tropical grasses, *Chloris* and *Panicum*. The yields of mixed plantings were found to be similar to those of grass monocultures over the first two years. However, by the third year the mixtures exceeded grass monocultures receiving annual applications of 50 kg ha^{-1} N. Stand longevity studies using rhizoma peanut/grass mixtures are unknown but there have been reports from established, mixed stands. For example, Santos et al. (2018) reported that 4-year-old, established rhizoma peanut/bahiagrass mixtures produced similar dry matter yields as grass monocultures receiving 90 kg N ha^{-1} in the first treatment year. However, in the second treatment year, the Argentine bahiagrass monoculture nearly doubled its yield compared to rhizoma peanut/grass mixtures. The increased bahiagrass production upon N fertilization suggests that bahiagrass may outcompete and reduce rhizoma peanut stands when there is adequate soil N fertility.

Rhizoma Peanut Establishment into Grasses

Although N fertilizer can often promote both, grass and legume growth, N applications during establishment may also fertilize the weeds. Minimizing weed competition with establishing rhizoma peanut as a monoculture or planted into bahiagrass swards, is challenging. There was lower weed frequency in Ecoturf rhizoma peanut plots than with other rhizoma peanut cultivars when planted into bermudagrass, which was due to its more rapid initial growth, but ‘UF Tito’ had fewer weeds by the second year of establishment (Mullenix et al., 2016). Weed control was more important than seed-bed preparation method for strip-planting rhizoma peanut into bahiagrass (Castillo et al., 2015). Planting into glyphosate-killed bahiagrass and managing with imazapic after rhizoma peanut emergence was an effective establishment practice. Castillo et al. (2015) reported a higher rate of rhizoma peanut sprout emergence in land that was tilled versus untilled. However, this type of land preparation requires large equipment that also will have limited maneuverability in small areas.

Establishment success also depends on planting date. The dormant period (January through March), resulted in nearly twice as many Florigraze sprouts per square meter compared to a summer planting (Williams, 1993). Mullenix et al. (2014) reported that at the end of the first growing season, Florigraze and Ecoturf rhizoma peanut produced greater coverage (30%) in the establishment year compared with other cultivars. The more decumbent growth habit of Ecoturf also protected it from intensive grazing/cutting. Hernandez-Garay et al. (2004) reported that established Florigraze in grass pastures persisted well under grazing pressure. However, Castillo et al. (2015) reported that intensive grazing or cutting of rhizoma peanut in a mixed bahiagrass sward may decrease rhizoma peanut canopy cover during establishment and over subsequent seasons.

Rhizoma Peanut-Bahiagrass Mixtures without N Inputs

Unlike a pasture or hay field, bahiagrass is intentionally not fertilized on Florida rights-of-way, due to costly fertilization and potentially extra mowing, along with concerns over increasing nutrient loss to the environment. There are few reports on the productivity of unfertilized, mixed rhizoma peanut/bahiagrass plantings. Jaramillo et al. (2018) reported Ecoturf–bahiagrass mixed plantings resulted in greater herbage accumulation than that of unfertilized ‘Pensacola’ bahiagrass, 4,160 and 2,710 kg ha^{-1}, respectively. In order to further assess the comparative performance and N fate of rhizoma peanut/bahiagrass mixtures for use as plantings for Florida rights-of-way, combinations of rhizoma peanut and bahiagrass cultivars were tested in plots at two Florida locations having different soil types.

The objective was to assess the compatibility of established rhizoma peanut grown with bahiagrass through 1) yield measurements, 2) determination of BNF using ^{15}N natural abundance, and 3) tracking soil pore water inorganic N.

Materials and Methods

Field design and locations

Field plots were established in 2011 through 2012 at North Florida Research and Education Center (NFREC), Quincy, FL and Gulf Coast Research and Education Center (GCREC), Wimauma, FL. Site location and soil descriptions are given in Chapter 2 (Fig. 3-1). Plots consisted of plant treatments with 3 replications (24 plots at 0.93 m^2 each). Alleys, (1.23 m wide), covered by black, plastic weed cloth, isolated each plot. Treatments were arranged in a randomized complete block design and consisted of 1) Argentine (ARG) monoculture, 2) experimental Dwarf F-9 (DF9) monoculture, 3) Ecoturf (ECO) monoculture, 4) experimental Quincy 6B (Q6b), 5) ARG/ECO, 6) ARG/Q6b, 7) DF9/ECO, and 8) DF9/Q6b. Argentine bahiagrass is commonly used in pastures and as utility turf in the southeastern United States, while DF9 was selected for its short canopy height and dense sward. The shorter canopy and seed head height makes it a potentially attractive alternative to Argentine, which is currently the preferred cultivar by FDOT. Ecoturf is characterized as more low-growing than the other commercially available rhizoma peanut options. The Q6B selection demonstrates resilience and competitive growth against common bermudagrass. It is also being considered for mixed pasture plantings.

Fig. 3-1. Field plots at NFREC and GCREC, 2014.

Field measurements

Porous cup lysimeters (one per plot) were installed in 2012, at both locations, at 90 cm depth. A vacuum was set on each lysimeter 48 hours prior to sample collection, and samples were collected approximately every three weeks. Each sample was measured for the total volume of soil pore water collected, filtered through qualitative filter paper, Fisher Q2 (Fisher Scientific, Waltham, MA) and then frozen until analysis. Inorganic N (NH_4-N and NO_3-N) were measured using the gas diffusion/conductivity method (model TL200, Timberline Instruments, Boulder, CO) and reported here as inorganic N.

Soil moisture was measured using a dielectric moisture sensor probe (AquaPro. Ducor, CA). Initial calibration of the probe was necessary to establish 0% (air) and 100% (water) moisture. Marks along the length of the probe allowed measurements at different depths when inserted into the polypropylene access tube (1 access tube per plot at NFREC only). Soil moisture was measured at 15, 22.5, 30, 45, 60, and 75 cm. Soil moisture was collected weekly from May 2013 to October 2014.

Sampling

A soil probe was used to collect initial samples (0 to 15 cm depth), as a composite (24 subsamples per composite) from each block. Soils were air-dried, passed through a 2 mm sieve and sent to a commercial lab (Waters Agricultural Laboratories, Camille GA) for M-3 extractable plant nutrients and pH (Table 2-1). Above ground biomass was collected in 2012, 2013 and 2014 at NFREC and in 2014 at GCREC. Only the 2014 data will be presented. Plots were harvested by hand within a 0.24 m^2 harvest square, at a 7.62 cm stubble height. All plots were mulched mowed after harvest, leaving the mulched clippings on the respective plots. Vegetation from mixed species plots were separated by hand into bahiagrass and rhizoma peanut components before sample fresh mass was recorded. Plant tissue samples were dried at 50^oC, then dry mass recorded. Tissue samples were milled to pass through a 2-mm sieve. Subsamples were ball milled, analyzed for C, N and ^{15}N, and Ndfa calculated, as described in Chapter 2 (Materials and Methods).

Data was analyzed using Proc Mixed from SAS 9.4 (SAS Inst., 2009). Fixed effect was Treatment. Block and interaction of block x treatment were considered random effects. The LSMEANS were compared using the PDIFF procedure adjusted for Tukey’s test. Differences were significant at $P \leq 0.05$.

Results and Discussion

Biomass and ^{15}N natural abundance

In 2014, yields were greatest for rhizoma peanut at both locations, at approximately 3,000 kg ha^{-1} (Figs. 3-2 a, b). Grass as monocultures or as grass/RP mixtures yielded about 50% less biomass. It is interesting to note that the RP provided no yield benefit over the grass monocultures at NFREC. In comparison, the mixtures at GCREC improved stand yield over grass monocultures (Fig. 3-2 b). Grass monocultures at GCREC were half the mass compared to the mixtures and grass treatments at NFREC. The percent RP biomass in mixed plantings were similar across treatments within each location. However, values averaged 30% rhizoma peanut at NFREC, compared with over 50% at GCREC. The RP at GCREC was visually appealing, while the grass looked N-stressed, lacking color and growth, particularly in the monoculture plots (Fig. 3-1).

In terms of BNF, the grass component (monocultures and mixed plantings) resulted in similarly low values (< 7 kg N ha^{-1}) at both locations (Fig. 3-3). As mentioned above, if the grass treatments had associative BNF, it was not contributing much N to the production. In comparison, BNF in the RP was over 65%, on average, resulting in N contributions of over 40 kg N ha^{-1} at NFREC and over 60 kg N ha^{-1} at GCREC (Figs. 3-3 c and d). Rhizoma peanut in mixtures and monocultures contributed similar BNF. The N contributions of monoculture ECO and Q6B were 41 and 42 kg N ha^{-1} harvest^{-1} respectively, and that of Santos et al. (2018) using the same plots, were comparable at 44 kg N ha^{-1} each. In comparison, Dubeux et al. (2017) reported in June 2014 that

Ecoturf BNF contributed near 50 kg N ha-1 at a field study at NFREC, Marianna (30.8500, $^{-}$85.1876). Only ‘UF Peace’ and ‘UF Tito’ contributed more. They did not test Q6B.

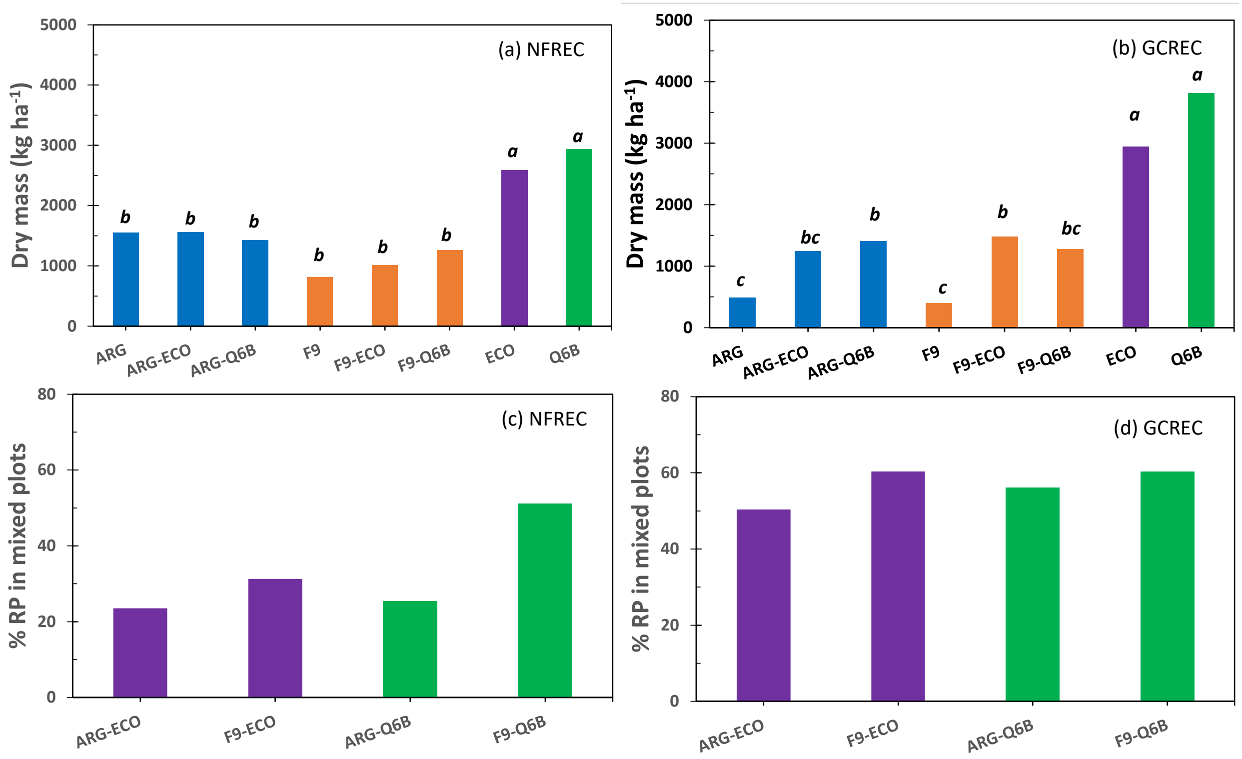

Fig. 3-2. Above-ground dry biomass from (a) NFREC and (b) GCREC, along with, percent rhizoma peanut in mixed plots collected at (c) NFREC and (d) GCREC. Each treatment represents the mean of three replicates. Bars sharing the same letter are not significantly different at α=0.05.

Soil δ^{15}N(‰) was 3.0 at NFREC and 3.7 at GCREC. These are somewhat low values and may suggest that using the ^{15}N natural abundance method for these tissues might add introduce some error into discriminating between legumes and grasses in some of our test soils.

Soil pore water N and soil moisture

Soil pore inorganic N was nearly always below 1 ppm through 2014. Values trended slightly higher at NFREC than GCREC but still below 1 ppm. At NFREC, RP, Q6B monoculture inorganic N averaged (0.17 ppm) ECO monoculture (0.10 ppm), and ARG and DF9 monocultures (0.8 ppm). These values fall well below values reported for agronomic systems relying on N inputs, including hay fields and pastures. For example, Woodard and Sollenberger (2011) reported lysimeter collection values of 9 and 98 ppm when bermudagrass hay fields were fertilized at either 126 or 168 kg N ha^{-1}.

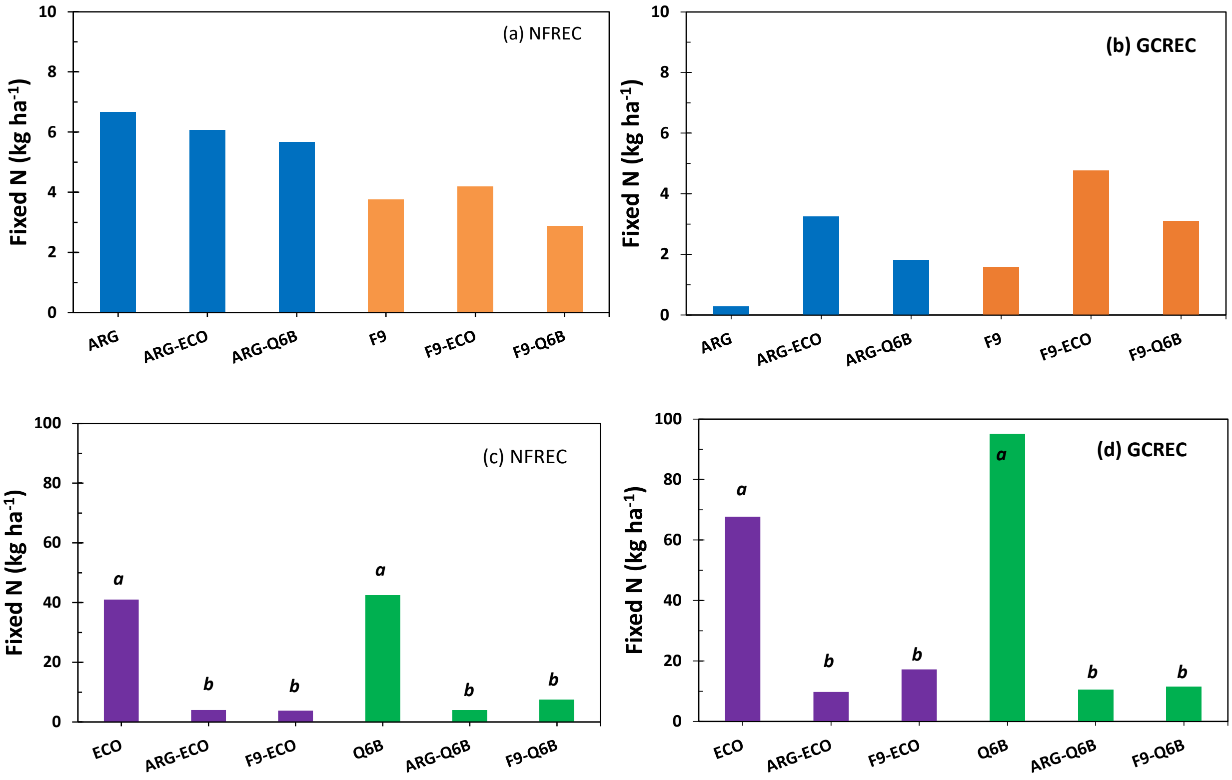

Fig. 3-4. Estimated fixed N (kg ha^{-1}) using ^{15}N natural abundance. The bahiagrass monoculture and bahiagrass component of mixed plots at (a) NFREC and (b) GCREC. Rhizoma peanut monoculture and component of mixed plots at (c) NFREC and (d) GCREC. Each treatment represents the mean of three replicates. Bars sharing the same letter are not significantly different at α=0.05.

The soil moisture measurements varied over the season, along with rainfall. In order to identify potential differences among cultivars, tracking drier periods helps delineate potential differences in water demand among treatments. October was a time of relatively low soil moisture in 2013. Soil moisture on 18 Oct resulted in ARG-Q6B and ARG-ECO demonstrating less soil moisture across depths, compared to Ecoturf monoculture (Fig. 3-4). Deeper and more extensive roots are able to capture water and sometimes nutrients at lower depths, since soil moisture typically increases with soil depth. For example, on 18 Oct, the NFREC plots, had significantly drier (16 to 20%) surface soil through 30 cm depth, while 60 to 76 cm depths held the most moisture (> 30%). Selecting plants with deeper root systems can benefit production. For example, grain yield in wheat crops benefitted from deeper rooting systems (Wood et al., 2012).

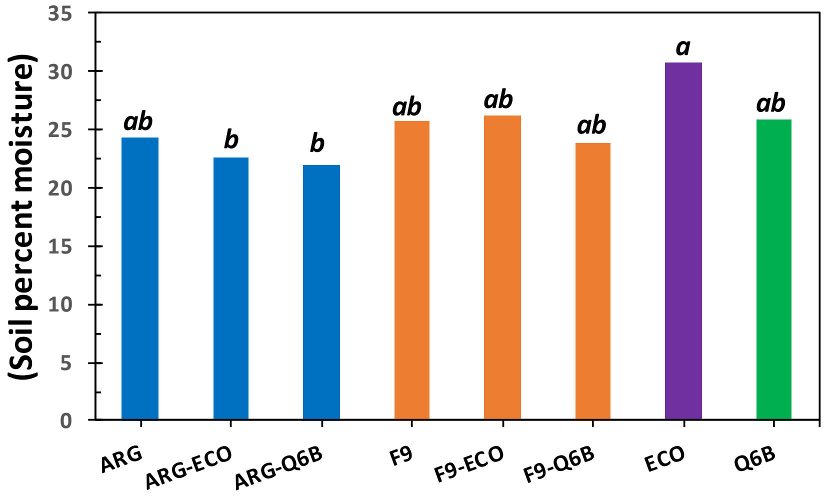

Fig. 3-4. Integrated soil moisture (percent of saturation: 50%) across depths from surface to 77 cm. Bars sharing the same letter are not significantly different at α=0.05.

Biomass and yield responses can vary by year and location. Developing multiple location trials allows for a better understanding of the range of responses within a test crop or production system. This may help increase the knowledge of a plant’s response to both, planned and unplanned conditions or events. For example, the mixed planting study results demonstrated some response differences between GCREC and NFREC. The grass production was much more limited at GCREC, and it appeared to be related to plant available N differences between the two locations. This was an unexpected result since soil TKN values were similarly low. These results reinforces the need to better understand reactive soil N and its impact on mixed legume/grass systems. At the same time, choosing only two locations may present too many dissimilar variables that are difficult to track or to be able to adequately interpret response differences from. Careful planning of treatments and the use of an appropriate experimental design makes it easier to address the hypothesis, while maybe also expanding our inquiry beyond what had originally been considered.

CHAPTER 4

RECOMMENDATIONS FOR USING RHIZOMA PEANUT IN RIGHTS-OF-WAY

Bahiagrass is one of the most common grasses found in Florida rights-of-way. It is a relatively inexpensive and low-maintenance plant that can provide erosion protection, while at the same time be an aesthetically pleasing groundcover. Lack of maintenance fertilization, aggressive mowing practices, and periodic disease pressure, are potential stresses that can decrease bahiagrass stand health and coverage. Declining stands open the soil surface to greater weed pressure and sloped land becomes more susceptible to erosion. Additionally, road maintenance budgets may benefit with groundcovers that require less frequent mowing.

Rhizoma peanut is a warm-season, perennial legume that is capable of BNF, and therefore, it is not reliant on N fertilizer inputs. It also originated from the same South American regions as bahiagrass, where the two species successfully co-exist. Incorporating rhizoma peanut into bahiagrass may provide the bahiagrass with a source of N through leaf litter, rhizome, root and nodule turn-over in the soil. The potential for disease caused stand decline in groundcovers of mixed species plantings is often less than in a bahiagrass monoculture. In general, the data and reports support the use of rhizoma peanut and bahiagrass in mixed stands, but good establishment and management practices are required.

Checklist for Establishing Rhizoma Peanut in Rights-of-way

Site Selection

Learning the soil type prior to planting is important for successfully establishing rhizoma peanut into bahiagrass. Florida soils (ultisols, spodosols, and entisols) are adequate for rhizoma peanut, except when the water table is near the soil surface, as is sometimes the case with certain spodosols. Additionally, rhizoma peanut is not a good option for areas prone to flooding, such as storm drainage or swales/ditches that are designed to collect or move water. Heavier ultisols, depending upon their land-use history, may have suitable fertility to support establishment without further amendments. Soil testing the potential site is recommended to address fertility and general suitability.

Site preparation is required, particularly when planting into an existing bahiagrass sod. To minimize competition with weeds and existing bahiagrass, consider using a non-selective herbicide, such as glyphosate. There are rules and regulations on herbicide selection and use for rhizoma peanut (Sellers and Ferrell, 2018). Pre-plant herbicide applications should be applied one to two weeks in advance to allow for better weed suppression and minimize the chance for residual activity that may harm the rhizoma peanut. Tilling or breaking the soil surface right before planting, if cost and location permit, is advantageous for faster establishment.

Planting

There are a few commercially available rhizoma peanut cultivars to choose from; however, Ecoturf has been quite successful in experimental establishment trials and propagation material is commercially available. Ecoturf combined with the experimental dwarf F9 bahiagrass tended to remove less water from the soil profile at a Quincy, FL trial. This might be an attractive plant mixture if further research supports initial observations. Additionally, the dwarf bahiagrass will require less mowing maintenance. For now, most of Florida's rights-of-way are planted in Argentine bahiagrass.

Rhizoma peanut planting material may be as rhizomes, plugs or sod. Each have advantages and disadvantages, and they may differ in terms of success, depending on the size and type of proposed planting area. Rhizome planting is the least expensive approach, especially for covering large areas. Rhizomes are typically planted in rows, approximately two inches beneath the soil surface. Rhizomes planted by this method with farm-scale equipment, is often limited to more level land, opposed to steep inclines/slopes. Heavy rains after planting may erode the slope, uncovering or washing away the rhizomes, thereby increasing the risk of poor coverage and lost rhizomes, unless preventative measures (netting, mulch, etc.) are taken. Vegetative plugs may be a better choice for steep slopes. While more expensive than rhizomes, whole plants may be used. If soil moisture is adequate and temperatures are moderate, the plugs establish more quickly than rhizomes. However, there will be bare patches between plants for most of the first season, depending upon planting density. Approximately one plug per 2 to 3 ft is often adequate. Sod is another option for establishing rhizoma peanut. This is, by far, the most expensive of the three options, but it often requires the least establishing time and the ground is covered from the start. Another disadvantage of sod is the initial lack of a functioning root system, which becomes an issue if the land is dry and has no access to irrigation. Therefore, supplemental irrigation must be available if this option is chosen.

The time of year to plant needs to be considered. Late winter through spring (February through April), when temperatures are moderate, soil moisture is often adequate, and water losses are low, is an optimal time to transplant. The more frequent, heavy rains (and tropical systems) during the summer months may not be the best time for rhizome plantings on inclines, due to the increased risk of erosion.

Maintenance

Some additional maintenance may be required after emergence, over the first season or two. Managing weeds with a recommended herbicide (Sellers and Ferrell, 2018) is important for ensuring greater rhizoma peanut spread early. Using a chemical mowing approach to suppress bahiagrass competition may be useful in areas where the bahiagrass growth is too aggressive. As long as N fertilizers are not applied, the soil N often declines to some extent, shifting the sward composition towards rhizoma peanut.

Future Research Questions

Incorporating a BNF plant, such as rhizoma peanut, into Florida rights-of-way might increase nitrate release into nearby, vulnerable waterways or groundwater. However, soil pore water samples (Chapter 3) demonstrated that NO_3-N leaching was typically below 1 mg L^{-1} (even though the rhizoma peanut cultivars trended higher than the grasses). More research is needed to verify that rhizoma peanut contributes little to no NO_3-N run-off or leaching loses to the environment, particularly if large-scale plantings are considered.

Further research on additional rhizoma peanut/bahiagrass cultivar combinations are needed to help expand their use under different landscapes and growing conditions.

Rhizoma peanut is slow to establish during the first year, but once established, it often competes well with bahiagrass in low-input, mixed plantings. Further research is required to help determine the role soil type has on rhizoma peanut BNF and rhizoma peanut/bahiagrass competitiveness for water and nutrients, particularly in terms of the system being an environmentally friendly option.

Research on the extended longevity of mixed rhizoma peanut/bahiagrass stands is needed to better assess return on investment for use with Florida rights-of-way. Based on the available data, rhizoma peanut/bahiagrass mixtures (particularly low-growing forms) should be given serious consideration as a sustainable planting option for addressing declining monoculture bahiagrass stands and a means for lowering maintenance costs on Florida rights-of-way.

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