Determining the Effects of St. Augustinegrass Cultivar Diversity on Belowground Ecosystem Processes

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ABSTRACT

Turfgrasses in the southeastern U.S. are produced and planted as cultivar monocultures. In recent years, PI Dale has investigated if this lack of genetic diversity predisposes lawns to insect pest attack, creating chronic pest pressure that increases insecticide use and plant replacement. The objective is to develop IPM tactics that improve lawns, meet industry needs, and reduce insecticide inputs. Thus far, PI Dale's lab has found that mixing any four cultivars of St. Augustinegrass in a single planting reduces insect pest pressure and may translate to agronomic benefits. Specifically, fall armyworm and southern chinch bug (two key pests of Florida turfgrasses) become less abundant and damaging in mixed-cultivar plantings compared to monocultures. Additionally, digital image analyses indicate that cultivar mixtures fill in and establish more quickly than cultivar monocultures after two years. A less frequently discussed, but critically important ecosystem service provided by turfgrass lawns is carbon storage. Soil carbon storage plays a vital role in mitigating anthropogenic increases in atmospheric CO2 concentrations and turfgrasses have great potential to enhance soil carbon storage. Research in grasslands and other natural systems has demonstrated that increasing plant diversity increases soil carbon storage by increasing belowground carbon inputs, soil organic matter, and soil microbial biodiversity and activity. Additionally, increasing microbial activity and diversity can increase decomposition, nutrient cycling, and other important soil processes, which improves overall plant health and quality. With 2019 FNGLA funding, we have found that increasing the number of St. Augustinegrass cultivars planted together increases the greenness and quality of turfgrass plantings. We are currently processing and collecting soil samples to see if this increase in quality is driven by changes in soil properties and processes. In addition to pest control benefits, cultivar mixtures of St. Augustinegrass may create a more ecologically functional soil ecosystems, enhancing the quality and value of turfgrass lawns.

OBJECTIVES AND METHODS

Objective 1. Determine the effect of mixing St. Augustinegrass cultivars on turfgrass lawn plant quality.

Objective 2. Determine the effect of mixing cultivars on soil properties, soil carbon storage, and soil microbial activity.

Objective 3. Determine if the effects of cultivar mixtures on belowground processes depends on geographic location.

To test the effects of cultivar diversity on turfgrasses and belowground processes, we created a regional common garden field experiment at four locations spanning six USDA Hardiness Zones. Specifically, we set up a replicated field experiment at UF IFAS Research stations in Ft. Lauderdale, Citra, and Jay, FL. We also established field plots at an NC State University facility that we are collaborating with in Jackson Springs, NC (**Figure 1**). For this study, we are only using the Florida locations. Differences in soil conditions coupled with substantial climatic variability among these sites will help us address Objective 3. To capture the effect of time over a longer period, we also utilized existing field research plots at the Citra, FL location that were planted in 2017 and follow a very similar experimental design.

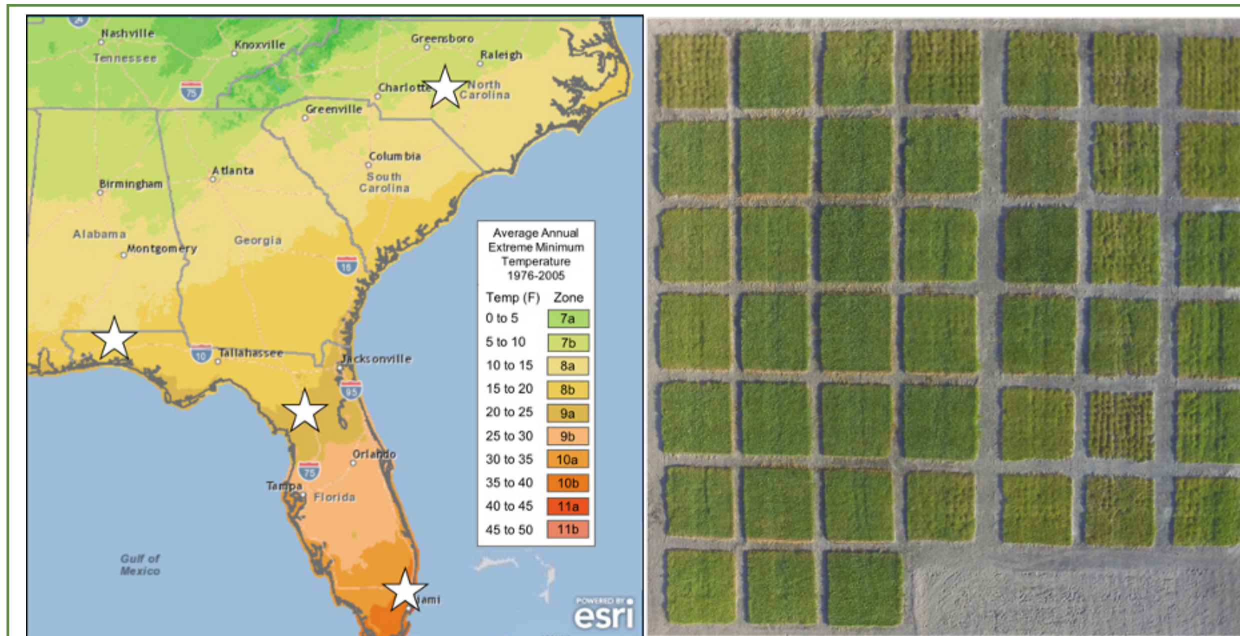

Figure 1. Field research plot locations (left) and aerial image of the field plot design (right).

We used St. Augustinegrass, *Stenotaphrum secundatum*, because it is the most commonly produced and planted warm season turfgrass in Florida and the southern U.S. We created three treatments at three different levels of cultivar diversity (low, medium, and high) using six of the most common commercially produced *S. secundatum* cultivars. The low diversity treatment consists of two or three replicates of all cultivars planted as monocultures (n=15). The medium diversity treatment consists of all combinations of two cultivars from the pool of six, resulting in 15 unique combinations (n=15). The high diversity treatment consists of all combinations of four cultivars from the pool of six, also resulting in 15 unique combinations (n=15). These treatments were planted in 3m x 3m plots as shown in Figure 1. Plots assigned to the same level of diversity vary in their cultivar composition, but we are testing for the effect of diversity, not composition, using the large plots. However, each monoculture and cultivar combination are replicated three times, resulting in 135 plots (45 M1, 45 M2, 45 M4) x 3 research sites for a total of N = 405 independent sampling units.

A potential effect of enhanced soil quality and activity is higher quality plant material. To address Objective 1 and determine if mixing St. Augustinegrass cultivars influenced turfgrass quality and marketability, we used three standardized methods to quantify turfgrass condition and appearance from a quantitative and qualitative perspective. First, we used the light box method to take close-up high resolution images of each plot for digital image analysis once per month. Funding from this project allowed us to compile a dataset of images taken on 23 separate months from May 2017 to December 2019 at the Citra, FL location. We analyzed each image using the Turf Analyzer software to capture quantitative measures of turfgrass color, density, texture, and quality. We also captured aerial drone images of the field plots on 25 separate months spanning April 2017 through November 2019 at the Citra, FL location. Drone images were analyzed using the Field Analyzer software and quantify percent green cover and dark green color index (DGCI) at the whole plot level on each date. We have also taken light box and drone images at our other field locations and will continue to do so monthly for the next 24 months.

To address Objective 2, we collect soil samples from within each field plot and process them to quantify soil properties, carbon, and microbial activity. Due to COVID-19 restrictions and limitations, and the departure of the graduate student working on this project, thus far we have only been able to collect soil samples from the Citra, FL field location. However, we still plan to collect soil samples from the Ft. Lauderdale and Jay locations before the end of 2020. Three soil subsamples will be collected per plot using a soil corer (5.5 cm in diameter) at 0-20 cm depth and homogenized as 300 g per plot.

We will partition each sample into smaller portions to capture soil properties and microbial activity. To investigate whether soil properties differed between cultivar diversity levels, we will quantify bulk density, soil macronutrients such as N, P, K, soil micronutrients such as Ca, Mg, Zn, Carbon:Nitrogen ratios, organic matter, pH, soil C and soil N.

We will extract microbial DNA from soil samples (0.3 g) using the MoBio PowerSoil DNA kit following the manufacturer's instructions. The abundance of 18S DNA will be measured by quantitative PCR (qPCR). For all samples, each 25 µL qPCR reaction contained 12.5 µL of PCR Master mix; 2 µL each of 10 µM forward and reverse primers; 3.5 µL of sterile, nuclease free water; and 5 µL of DNA sample. Melting curve analyses and agarose gel electrophoresis will be used to confirm that the fluorescence signal resulted from the specific PCR products. Also, amplicons will be visualized on agarose gel containing SYBR dye on illuminator. The qPCR assays will be conducted in 96-well plates with two replicates. Amplicons will be sequenced, and then the sequencing data will be processes for analysis using USEARCH v9.2.64, QIIME v1.9.1, RStudio v3.4.3.

To date – we are still processing or waiting to process soil samples to quantify soil properties and microbial DNA.

RESULTS

As anticipated, turfgrass quality metrics changed over time, initially as plots established and plant material grew in, but also as various biotic and abiotic factors influenced plant traits within each plot. As St. Augustinegrass cultivar diversity increased, percent green cover, DGCI, color quality, and overall quality increased. On average across 23 survey dates, plots containing two or four cultivars had statistically greater percent green cover, color quality, and overall quality than plot containing only one cultivar (**Table 1**). In some cases, mixtures of four cultivars were superior to both monocultures and mixtures of two cultivars. For example, on average across all dates, mixtures of four cultivars had DGCI and color quality values greater than monocultures and mixtures of two cultivars (**Table 1**). As time progressed, particularly in 2019, the difference between cultivar blends and monocultures increased. This trend was apparent in overall quality ratings, which were statistically higher on average in both mixed cultivar treatments compared to monocultures (**Figure 1**).

As in the light box image analyses, differences in percent green cover between cultivar diversity levels became more pronounced with time, particularly in 2019. In contrast to light box image analyses, average percent green cover and DGCI across 25 dates was no different between mixtures of two cultivars and cultivar monocultures (**Table 2**). However, mixtures of four cultivars averaged statistically greater percent green cover than monocultures and greater DGCI than monocultures and mixtures of two cultivars (**Table 2**).

Due to COVID-19 and the departure of the graduate student working on this project, we are currently processing or waiting to process soil samples to quantify soil properties and microbial DNA. We anticipate these being completed by the beginning of 2021.

Table 1. Lightbox image analysis results. Turfgrass quality metrics were captured once per plot (n=15 per treatment) on 23 dates spanning May 2017 through December 2019.

Metric	Factor	Treatment	LS Means*	F	P
Percent green cover	Diversity			$11.59_{2,995}$	<0.0001
		Monoculture	65.06 b		
		Mix of two	67.82 a		
		Mix of four	68.79 a		
	Date			$145.42_{22,995}$	<0.0001
Dark Green Color Index (DGCI)	Diversity			$12.48_{2,995}$	<0.0001
		Monoculture	0.51 b		
		Mix of two	0.52 b		
		Mix of four	0.53 a		
	Date			$106.37_{22,995}$	<0.0001
Color Quality	Diversity			$14.94_{2,995}$	<0.0001
		Monoculture	5.01 c		
		Mix of two	5.30 b		
		Mix of four	5.63 a		
	Date			$8.96_{22,995}$	<0.0001
Overall Quality	Diversity			$10.04_{2,995}$	<0.0001
		Monoculture	5.59 b		
		Mix of two	5.76 a		
		Mix of four	5.88 a		
	Date			$13.36_{22,995}$	<0.0001

*Different letters next to values indicate statistical differences between means using Tukey HSD

Table 2. Aerial drone image analysis results. Photos taken once per month on 25 dates spanning April 2017 through November 2019.

Metric	Factor	Treatment	LS Means*	F	P
Percent green cover	Diversity			$3.41_{2,1084}$	0.03
		Monoculture	0.97 b		
		Mix of two	0.98 ab		
		Mix of four	0.99 a		
	Date			$17.12_{24,1084}$	<0.0001
Dark Green Color Index (DGCI)	Diversity			$6.37_{2,1084}$	0.002
		Monoculture	0.435 b		
		Mix of two	0.437 b		
		Mix of four	0.442 a		
	Date			$86.59_{24,1084}$	<0.0001

*Different letters next to values indicate statistical differences between means using Tukey HSD

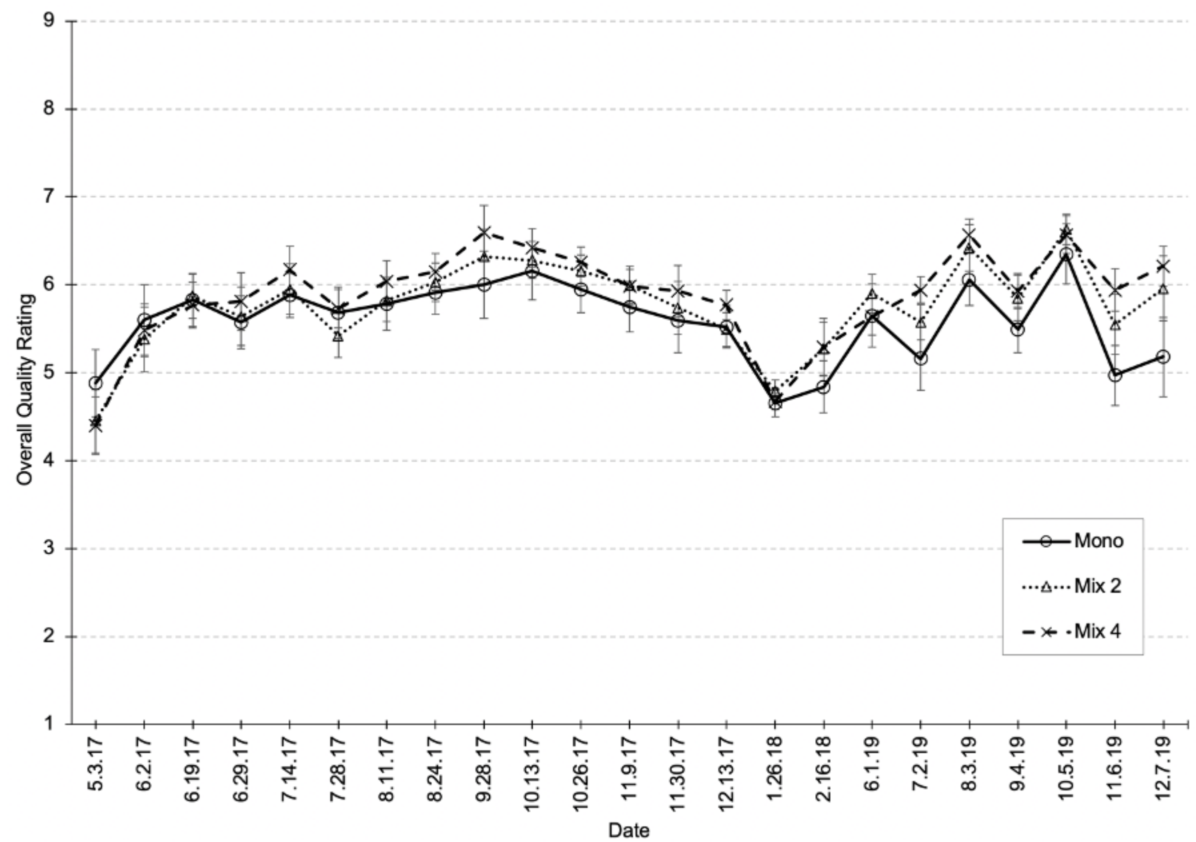

Figure 2. Overall Quality Rating based on the weighted averages of multiple turfgrass agronomic traits calculated using lightbox photos. Different lines indicate cultivar diversity treatments: Monocultures, Mixtures of two, and Mixtures of four cultivars. Photos taken once per plot (n=15 per treatment) on 23 dates spanning May 3, 2017 through December 7, 2019.

CONCLUSIONS

Based on our results thus far, we have found evidence that increasing the number of St. Augustinegrass cultivars planted together increases the greenness and quality of plant material. This may be due to several mechanisms, but a few of those relate to soil quality, nutrient availability and uptake from the soil, and soil microbial activity. Ongoing work will hone in on the mechanisms driving our observed differences in plant quality and should shed light on whether mixing St. Augustinegrass cultivars affects soil properties and/or microbial activity. To-date, we have documented benefits of mixing St. Augustinegrass cultivars on insect pest management and turfgrass plant quality and performance. If we find benefits of cultivar mixtures on soil properties and processes, it would provide additional evidence that cultivar blends of St. Augustinegrass may be a superior method for producing, planting, and maintaining warm season turfgrass lawns. Ongoing ancillary research is investigating the effects of cultivar mixtures on key insect pests and weeds to provide more comprehensive evidence of the effects of cultivar diversity on turfgrass pests. Although we have captured three consecutive years of field observations, additional research will be needed to evaluate how this approach would translate to sod production and long-term installation and maintenance in residential lawns.