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Bermudagrass Establishment, Aesthetics, and Function in Response to the Microbial Inoculant *Klebsiella variicola* and Fertilizer Timing

Connor Bolton and Miguel Cabrera
Department of Crop and Soil Sciences, University of Georgia, 3111 Miller Plant Science Building, Athens, GA 30602

Mussie Habteselassie
Department of Crop and Soil Sciences, University of Georgia, 1109 Experiment Street, Griffin, GA 30223

Daniel Poston
Pivot Bio, 2910 7th Street, Berkley CA 94710

Gerald Henry
Department of Crop and Soil Sciences, University of Georgia, 3111 Miller Plant Science Building, Athens, GA 30602

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Abstract. **The incorporation of biostimulants, including microbial inoculants, into turfgrass management programs has increased during the past decade as a result of the potential benefits of their use, including increased nutrient uptake, enhanced growth, and improved stress tolerance. However, minimal research has been conducted on warm-season grasses, and questions still exist regarding microbial inoculant application timing, frequency of inoculation, and need for supplemental nitrogen. Therefore, the objective of our research was to investigate the influence of nitrogen fertilizer and microbial inoculant application timings on the establishment of common bermudagrass [*Cynodon dactylon* (L.) Pers.] in the field and in a controlled environment. Treatments containing fertilizer had a consistently greater normalized difference vegetation index, turfgrass color, and turfgrass quality than treatments that contained only the *Klebsiella variicola* microbial inoculant (KV). Establishment of bermudagrass plots in response to treatments containing fertilizer had more than 90% cover at the conclusion of the study (8 weeks after seeding), whereas KV treatments and the untreated check never exceeded 70% cover. However, the greatest change in carbon efflux was often observed in the field in response to treatments that supplied KV 3 weeks after seeding. In the greenhouse, the greatest root and shoot weights were typically observed in response to treatments containing fertilizer, whereas KV-alone treatments resulted in root and shoot weights either similar to or less than the untreated check.**

Supplemental nitrogen (N) applications are often required for turfgrass to achieve optimal performance because N is typically the most deficient macronutrient in a turfgrass system (Bauer et al., 2012; Frank and Guertal, 2013; Mills and Jones, 1997; Turner and Hummel, 1992; Walker and Branham, 2020). Fertilization with N is important for both turfgrass growth and metabolism. N fertility promotes the formation of a dense, resilient canopy that functions as an aesthetic landscape, a safe playing surface, and a productive ecosystem (Carrow et al., 2002; Christians et al., 2016; Frank and Guertal, 2013). As a component of numerous plant biochemical constituents that include chlorophyll, amino acids, and enzymes, accessibility to N will ultimately determine the persistence and health of a turfgrass system (Carrow et al., 2002; Christians et al., 2016; Frank and Guertal, 2013; Marschner, 2011). However, the application and production of synthetic N fertilizers can have a negative impact on the same environment they are intended to benefit.

Synthetic ammonium nitrate and urea fertilizers are created from ammonia that is generated during the artificial industrial N fixation procedure known as the Haber-Bosch process (Kandemir et al., 2013; Kyriakou et al., 2020; Smith et al., 2020; Xu et al., 2019). This technique perpetuates the reaction of naturally abundant atmospheric N with hydrogen to synthesize ammonia directly (Amin et al., 2013; Jennings, 1991; Kandemir et al., 2013; Modak, 2002). The production of ammonia using the Haber-Bosch process consumes ≈1% to 2% of the global energy while emitting 1.2% of carbon dioxide (CO_2) emissions (Kyriakou et al., 2020; Smith et al., 2020). Furthermore, the high solubility of synthetic fertilizers can affect the environment negatively through N leaching/runoff and subsequent contamination of groundwater and eutrophication of lakes and streams (Frank and Guertal, 2013; Mulvaney et al., 2009; Shuman, 2002; Walker and Branham, 2020).

Previous research has attempted to investigate alternatives to synthetic N fertilizer applications in an effort to reduce potential negative environmental impacts and enhance turfgrass sustainability. Microbial and nonmicrobial products intended to improve turfgrass establishment and growth, increase tolerance to environmental stress, and augment plant nutrition have collectively been categorized as biostimulants (Brown and Saa, 2015; Calvo et al., 2014; Du Jardin, 2015; Rouphael and Colla, 2020; Yakhin et al., 2017). However, minimal research has addressed the use of microbial inoculants in turfgrass systems, and findings have been inconsistent to date. Increases in perennial ryegrass (*Lolium perenne* L.) and tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] color and clipping yield were observed by Acikgoz et al. (2016) in response to N-fixing bacteria (*Bacillus* spp.). De Luca et al. (2020) reported similar improvements in perennial ryegrass quality when treated with several N-fixing bacteria (*Azotobacter*, *Bacillus*, and *Pseudomonas* spp.). Contrarily, Peacock and Daniel (1992) could not discern any enhancements in growth, N uptake, or disease suppression of tall fescue and hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt Davy] after *Bacillus* spp. inoculation.

Klebsiella variicola is a growth-promoting bacteria that has been observed to colonize the rhizosphere and potentially improve agricultural production (Lin et al., 2015; Pinto-Tomas et al., 2009; Yang and Yang, 2020). Sangabriel-Conde et al. (2015) reported a 28% increase in rice (*Oryza sativa* L.) yield in response to *K. variicola*, whereas Yang and Yang (2020) noted substantial increases in soil nutrients (N, phosphorus, and potassium) and enzymes within the rhizosphere of corn (*Zea mays* L.) seedlings. Although a handful of studies have evaluated the benefits of *K. variicola* in cropping systems, no published research has examined its use in turfgrass environments.

The production of a dense canopy and tolerance to an array of environmental stresses including heat, drought, and traffic make bermudagrass (*Cynodon* spp.) one of the most widely used turfgrass species in the southeastern region of the United States (Christians et al., 2016; Emmons and Rossi, 2015; Hanna

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G.H. is the corresponding author. E-mail: gmhenry@uga.edu.

et al., 2013; Taliaferro et al., 2004). Versatility and affordability of establishment—whether from seed, sprigs, or sod—make common bermudagrass [*Cynodon dactylon* (L.) Pers.] a popular choice for home lawns and low-input areas (Chalmers et al., 2006; Rice et al., 2019; Taliaferro et al., 2004). Significant soil disturbance may occur before turfgrass establishment, often resulting in the removal of topsoil. Therefore, N applications may be necessary during establishment in these depleted soils, although starter fertilizers are typically low in N and high in phosphorus content.

Investigation into the use of N-fixing bacteria in turfgrass management has been limited, with most research conducted in the greenhouse and focused on cool-season turfgrass species (Aamlid and Hanslin, 2009; Acikgoz et al., 2016; DeLuca et al., 2020). Uncertainties still exist regarding application timing and need for supplemental N or sequential inoculant applications. Furthermore, research evaluating the response of turfgrass to the bacteria *K. variicola* has not been documented. Therefore, the objective of our research was to investigate the influence of N fertilizer and *K. variicola* application timings on the establishment of common bermudagrass in the field and in a controlled environment.

Materials and Methods

Field experiments. Experiments were conducted at the Athens Turfgrass Research and Education Center (ATREC) in Athens, GA (lat. 33.54°N, long. 83.22°W) and at the University of Georgia Facilities Maintenance Division (FMD) complex in Athens, GA (lat. 33.92°N, long. 83.37°W). The soil at ATREC was a Cecil sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludults) with a pH of 5.5 and an organic matter content of 1.2%, whereas the soil at FMD was a Cecil sandy clay loam with a pH of 5.9 and an organic matter content of 1.7%. Seedbed preparation at ATREC consisted of cultivating the research site in two directions (perpendicular to one another) with a tractor-mounted rototiller (Bush Hog, Selma, AL) to a depth of 10.2 cm, and grading to provide a smooth planting bed of desired contour. A hand tiller (Stihl, Waiblingen, Germany) was used to a depth of 10.2 cm at the FMD location before grading to prepare the seedbed for planting. Plots measured 1.5 × 1.5 m and were arranged in a randomized complete block design with four replications at both locations.

'Sahara II' common bermudagrass (uncoated/untreated) was seeded at 98 kg·ha^{-1} on 28 July 2021 at both locations. A seeding box (1.5 × 1.5 m) was placed on the corners of each plot, and seed was applied evenly across the soil surface using a shaker jar. Treatments were applied immediately to the soil after seeding and consisted of no treatment, fertilizer (5N–5P_2O_5–5K_2O) (EarthWorks Natural Organic Products, Martins Creek, PA) at 24 kg·ha^{-1} N at seeding, fertilizer at seeding + 3 weeks after seeding (WAS), *K. variicola* 137-1036 (KV) at an inoculant rate of 1.87 L·ha^{-1} [4 × 10^8 colony-forming units (cfu)/mL] and a carrier volume of 3000 L·ha^{-1} at seeding, KV at seeding + 3 WAS, fertilizer at seeding + KV 3 WAS, and fertilizer + KV at seeding + KV 3 WAS. Fertilizer was applied by hand using a shaker jar. Microbial inoculants were applied with distilled water using a watering can. Plots received ≈0.3 cm of water through an overhead irrigation system after treatment application and were covered with germination cloth (A.M. Leonard, Inc., Piqua, OH) to promote seed germination and prevent desiccation. Plants were mowed weekly (starting at 4 WAS) to a height of 3.81 cm with a walk-behind rotary mower (American Honda Motor Co., Alpharetta, GA), with clippings collected and removed. Clippings were removed to avoid interference with grid count data collection. About 2.5 to 4 cm of water per week was applied at ATREC and FMD through an overhead irrigation system (well water).

Turfgrass color (TC), turfgrass quality (TQ), and normalized difference vegetation index (NDVI) were recorded at trial initiation and 3, 5, and 8 WAS. Visual ratings of TC and TQ were recorded on a scale of 1 to 9, with a rating of 6 considered acceptable TC and TQ (Morris and Shearman, 2007). NDVI was recorded with a Field Scout CM 1000 NDVI chlorophyll meter (Spectrum Technologies Inc., Aurora, IL). A vegetative index (range, 0–1; where 1 is best) was calculated from the reflectance readings as follows: NDVI = (R770 – R660)/(R770 + R660). An average of three readings was obtained per plot per rating date. Grid counts were conducted to assess bermudagrass cover at 3, 5, and 8 WAS. A 0.3-m^2 grid with 2.5- × 2.5-cm intersect spacing was placed randomly within each plot. The following equation was used to convert grid counts to percent cover:

$$\left(\frac{a}{b}\right) \times 100 = c, \qquad [1]$$

where a is the number of intersects where bermudagrass was present, b is the total number of intersections (25), and c is percentage of turfgrass cover (Richardson et al., 2001).

Measurements of CO_2 efflux (CE) (measured in micromoles per square meter per second) were recorded at trial initiation and 5 and 8 WAS with a LI-COR 8100 automated system (LI-COR, Inc., Lincoln, NE) to provide plant root and soil microbial respiration as well as to determine the overall metabolic activity of each system (Jensen et al., 1996; Ryan and Law, 2005). The setup included an infrared gas analyzer connected to the LI-COR 8100 device via an RS-232 serial cable. The LI-COR 8100 was also connected to a laptop computer via an ethernet cable to run the LI-8100A 4.0.0 software for sample collection. A 20-cm infrared gas analyzer survey chamber was placed on top of a polyvinyl chloride collar (diameter, 20 cm) that was inserted randomly into each plot to contain the sampling area (offset, 7.5 cm) for measurement. To prevent any irrelevant CO_2 buildup in the chamber, a 60-s prepurge and 45-s postpurge was conducted before and after each measurement, respectively. After closing the chamber, a dead-band period of 40 s was used before measurements were initiated to obtain a constant rate of efflux. An observation length of 60 s was used for each measurement. Change over time (Δ) for CE was determined for 5 and 8 WAS by comparing values to initial measurements.

Greenhouse experiments. Experiments were conducted at the ATREC greenhouse complex (lat. 33.54°N, long. 83.22°W) in Athens, GA, during Summer 2021. On 27 July 2021, 'Sahara II' common bermudagrass (uncoated/untreated) was seeded at a rate of 98 kg·ha^{-1} into circular pots (diameter, 15.2 cm) containing a 2:1 mixture of Cecil sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludults) and Wakulla sand (siliceous, thermic Psammentic Hapludults). Seed was applied evenly to the soil surface and top-dressed lightly to increase seed-to-soil contact and reduce desiccation. Two pots were established per treatment per experiment replication to conduct a time-lapse destructive harvest at 3 and 6 WAS. Two experimental runs were conducted simultaneously in separate greenhouses using a randomized complete block design with five replications.

Treatments were applied on 27 July 2021 and were the same as described previously for field experiments. Fertilizer (5N–5P_2O_5–5K_2O) was applied by hand using a shaker jar at a rate of 24 kg·ha^{-1} N. A stock solution of the microbial inoculant KV was prepared with distilled water and applied to the soil with a 50-mL syringe at an inoculation rate of 1.87 L·ha^{-1} (4 × 10^8 cfu/mL) and a carrier volume of 3000 L·ha^{-1}. Sequential applications of treatments were made on 17 Aug. 2021. Pots were irrigated immediately after inoculant and fertilizer applications with ≈0.3 cm of water to incorporate treatments into the soil. An untreated check was included for comparison. The pots was watered using an overhead irrigation system calibrated to deliver 3.8 cm of water/week. Natural light was supplemented with artificial light (metal halide) to remain at 500 μmol·m^{-2}·s^{-1} photosynthetic photon flux (measured at the canopy) in a 12-h day to approximate summer light intensity and photoperiod. Conditions in the climate-controlled greenhouse were maintained at day/night temperatures of 32 °C/26 °C. Experimental blocks were arranged along a gradient created by the greenhouse cooling pads and associated fans.

Pots were harvested destructively at 3 and 6 WAS. Roots and shoots were separated from each other, washed of all soil, dried in an oven for 48 h at 110 °C, and weighed to determine root and shoot biomass (in grams) at 3 and 6 WAS.

Data analysis. Analysis was conducted separately for TC, TQ, NDVI, and percentage of turfgrass cover at 3, 5, and 8 WAS, as well as CE and ΔCE at 5 and 8 WAS for field experiments. In addition, a separate analysis was also conducted for root and shoot weights at 3 and 6 WAS for greenhouse experiments. Analysis of variance was performed using PROC MIXED with the appropriate expected mean square values described by McIntosh (1983) in SAS (SAS version 9.2 for Windows;

Table 1. Establishment (percentage of turfgrass cover) of seeded 'Sahara II' common bermudagrass [*Cynodon dactylon* (L.) Pers.] in response to fertility and microbial inoculants applied during Summer 2021 in Athens, GA.

	Turfgrass cover[y] (%)		
Treatment[z]	3 WAS	5 WAS	8 WAS
Untreated check	23.0 d[x]	41.0 c	65.0 b
F at seeding	53.0 b	63.0 b	89.5 a
F at seeding + 3 WAS	48.5 b	77.0 a	99.0 a
KV at seeding	25.5 cd	42.0 c	59.0 b
KV at seeding + 3 WAS	33.5 c	47.0 c	68.5 b
F at seeding + KV 3 WAS	56.0 ab	67.0 ab	88.5 a
F + KV at seeding + KV 3 WAS	64.5 a	76.0 a	95.0 a
$LSD_{(0.05)}$	10.2	11.1	13.6

[z]Fertilizer was applied by hand using a shaker jar at a rate of 24 kg·ha^{-1} nitrogen. Microbial inoculants were applied with a watering can using distilled water at an inoculation rate of 1.87 L·ha^{-1} (4×10^8 CFU/mL) and a carrier volume of 3000 L·ha^{-1}.

[y]Percentage of turfgrass cover was assessed by randomly placing a 0.3-m^2 grid with 2.5- × 2.5-cm intersect spacing (25 intersections) in the middle of each plot.

[x]Means within a column followed by the same lowercase letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

F = fertilizer (5N–5P_2O_5–5K_2O); KV = microbial inoculant containing *Klebsiella variicola*; $LSD_{(0.05)}$ = least significant difference at $P \leq 0.05$; WAS = weeks after seeding.

SAS Institute, Cary, NC). Means were separated according to Fisher's protected least significant difference test at $\alpha = 0.05$. Data were arcsine square root–transformed to stabilize variance as described by Bowley (2008). Transformed and nontransformed data were analyzed, and interpretations were not different; therefore, nontransformed means are presented for clarity.

Results and Discussion

Field experiments. Experimental run-by-treatment interactions for field experiments were not significant ($P = 0.57$). Therefore, data from individual runs were pooled and are presented accordingly. At 3 WAS, the greatest percentage of turfgrass cover was observed in response to fertilizer + KV at seeding (64.5%) followed by fertilizer at seeding (48.5%– 56%; Table 1). The microbial inoculant applied alone at seeding resulted in 25.5% to 33.5% cover, which was slightly greater than the untreated check 3 WAS (23%). Sequential applications of fertilizer, fertilizer + KV at seeding + KV 3 WAS, and fertilizer at seeding + KV 3 WAS exhibited the greatest percentage of turfgrass cover 5 WAS (77%, 76%, and 67%, respectively; Table 1). The single application of fertilizer at seeding resulted in slightly less of a percentage of turfgrass cover 5 WAS (63%), whereas sequential applications of KV, KV at seeding, and the untreated check were statistically similar (47%, 42%, and 41%, respectively). At 8 WAS, all treatments that received at least one application of fertilizer resulted in a similar percentage of turfgrass cover (88.5%–99%), whereas treatments that consisted only of KV (59%–68.5%) had similar cover as the untreated check (65%; Table 1).

At 3 WAS, the greatest NDVI (0.52–0.58) and TQ (3.6–4.1) ratings were observed in response to treatments that supplied fertilizer at seeding (Table 2). Three of the four fertilizers-at-seeding treatments also resulted in the greatest TC (5.1–5.6). The microbial inoculant applied alone at seeding resulted in a similar NDVI (0.38–0.39), TC (4.1–4.3), and TQ (2.7–3.1) as the untreated check (0.36, 0.39, and 2.6, respectively). Sequential fertilizer applications, fertilizer + KV at seeding + KV 3 WAS, fertilizer at seeding + KV 3 WAS, and fertilizer at seeding treatments resulted in the greatest NDVI (0.60–0.65) and TC (5.6–6.1) 5 WAS (Table 2). The greatest TQ 5 WAS also observed in response to sequential fertilizer applications (6.1), fertilizer + KV at seeding + KV 3 WAS (6.1), and fertilizer at seeding + KV 3 WAS (5.8). At 5 WAS, KV-alone treatments resulted in a similar NDVI (0.51–0.54), TC (4.6–4.7), and TQ (3.6–3.9) as the untreated check (0.50, 4.7, and 3.4, respectively). A similar trend was observed 8 WAS with the greatest NDVI, TC, and TQ in response to sequential fertilizer applications, fertilizer + KV at seeding + KV 3 WAS, fertilizer at seeding + KV 3 WAS, and fertilizer at seeding (Table 2). Treatments receiving KV alone still resulted in similar NDVI, TC, and TQ as the untreated check 8 WAS.

Treatments receiving KV 3 WAS resulted in the greatest ΔCE 5 WAS, with fertilizer at seeding + KV 3 WAS exhibiting the greatest ΔCE (5.3 μmol·m^{-2}·s^{-1}; Table 3). The microbial inoculant at seeding (3.4 μmol·m^{-2}·s^{-1}) and treatments only supplying fertilizer (3.1 μmol·m^{-2}·s^{-1}) were statistically similar to the ΔCE of the untreated check (2.1 μmol·m^{-2}·s^{-1}). At 8 WAS, fertilizer at seeding + KV 3 WAS and fertilizer + KV at seeding + KV 3 WAS resulted in the greatest ΔCE (4.5–4.7 μmol·m^{-2}·s^{-1}); however, the ΔCE of the untreated check (3.2 μmol·m^{-2}·s^{-1}) was statistically similar (Table 3). All other treatments exhibited ΔCE ≤ 2.8 μmol·m^{-2}·s^{-1}.

Greenhouse experiments. Experimental run-by-treatment interactions for greenhouse experiments were significant ($P = 0.03$). Therefore, data were not pooled across experimental runs and results for each experiment are presented separately.

In experimental run 1, treatments that contained initial fertilizer applied alone exhibited

Table 2. Normalized difference vegetation index (NDVI), turfgrass color (TC), and turfgrass quality (TQ) of seeded 'Sahara II' common bermudagrass [*Cynodon dactylon* (L.) Pers.] in response to fertility and microbial inoculants applied during Summer 2021 in Athens, GA.

	3 WAS			5 WAS			8 WAS		
Treatment[z]	NDVI[y]	TC	TQ	NDVI	TC	TQ	NDVI	TC	TQ
Untreated check	0.36 b[x]	3.9 d	2.6 c	0.50 c	4.7 b	3.4 c	0.56 c	4.9 c	4.4 c
F at seeding	0.52 a	4.9 bc	3.6 ab	0.60 ab	5.6 a	4.6 b	0.65 ab	6.0 b	5.6 b
F at seeding + 3 WAS	0.52 a	5.1 a	4.0 a	0.65 a	6.1 a	5.1 a	0.7 a	6.6 a	6.5 a
KV at seeding	0.38 b	4.1 d	2.7 c	0.51 c	4.6 b	3.6 c	0.61 bc	5.1 c	4.3 c
KV at seeding + 3 WAS	0.39 b	4.3 cd	3.1 bc	0.54 bc	4.7 b	3.9 c	0.61 bc	5.3 c	4.6 c
F at seeding + KV 3 WAS	0.52 a	5.1 ab	4.0 a	0.63 a	5.8 a	4.9 ab	0.69 a	6.2 ab	5.8 ab
F + KV at seeding + KV 3 WAS	0.58 a	5.6 a	4.1 a	0.65 a	6.1 a	5.2 a	0.69 a	6.3 ab	6.0 ab
$LSD_{(0.05)}$	0.09	0.6	0.6	0.06	0.6	0.6	0.07	0.6	0.7

[z]Fertilizer was applied by hand using a shaker jar at a rate of 24 kg·ha^{-1} nitrogen. Microbial inoculants were applied with a watering can using distilled water at an inoculation rate of 1.87 L·ha^{-1} (4×10^8 CFU/mL) and a carrier volume of 3000 L·ha^{-1}.

[y]NDVI was recorded with a Field Scout CM 1000 NDVI chlorophyll meter. A vegetative index (range, 0–1; where 1 is best) was calculated from the reflectance readings as follows: NDVI = (R770 – R660)/(R770 + R660). An average of three readings were obtained per plot per rating date. Visual ratings of TC and TQ were recorded on a scale of 1 to 9, with a rating of 6 considered acceptable TC and TQ.

[x]Means within a column followed by the same lowercase letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

F = fertilizer (5N–5P_2O_5–5K_2O); KV = microbial inoculant containing *Klebsiella variicola*; $LSD_{(0.05)}$ = least significant difference at $P \leq 0.05$; WAS = weeks after seeding.

Table 3. Carbon efflux of seeded 'Sahara II' common bermudagrass [*Cynodon dactylon* (L.) Pers.] in response to fertility and microbial inoculants applied during Summer 2021 in Athens, GA.

	Carbon efflux ($\mu mol \cdot m^{-2} \cdot s^{-1}$)[y]			
Treatment[z]	5 WAS	Δ5 WAS	8 WAS	Δ8 WAS
Untreated check	4.7 c[x]	2.1 c	5.8 ab	3.2 ab
F at seeding	7.7 ab	3.1 bc	6.5 ab	1.9 b
F at seeding + 3 WAS	8.1 a	3.1 bc	6.8 ab	1.8 b
KV at seeding	6.3 b	3.4 bc	5.8 ab	2.8 b
KV at seeding + 3 WAS	8.1 a	4.7 ab	5.5 b	2.1 b
F at seeding + KV 3 WAS	8.0 a	5.3 a	7.2 a	4.5 a
F + KV at seeding + KV 3 WAS	6.8 ab	4.4 ab	7.1 a	4.7 a
$LSD_{(0.05)}$	1.4	1.7	1.5	1.7

[z]Fertilizer was applied by hand using a shaker jar at a rate of 24 $kg \cdot ha^{-1}$ nitrogen. Microbial inoculants were applied with a watering can using distilled water at an inoculation rate of 1.87 $L \cdot ha^{-1}$ (4×10^8 CFU/mL) and a carrier volume of 3000 $L \cdot ha^{-1}$.

[y]Carbon efflux measurements were recorded with a LI-COR 8100A automated system at trial initiation as well as 5 and 8 WAS to provide insight into plant and soil microbial activity (respiration). Data recorded at 5 and 8 WAS were compared with data recorded at trial initiation to calculate carbon efflux change over time (Δ).

[x]Means within a column followed by the same lowercase letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

F = fertilizer ($5N–5P_2O_5–5K_2O$); KV = microbial inoculant containing *Klebsiella variicola*; $LSD_{(0.05)}$ = least significant difference at $P \leq 0.05$; WAS = weeks after seeding.

the greatest root weights (0.43–0.56 g) 3 WAS (Table 4). Treatments that contained initial KV applications resulted in the lowest root weights (0.32–0.41 g) and were statistically similar to the untreated check (0.28 g) 3 WAS. The greatest shoot weights (1.15–1.45 g) 3 WAS were observed in response to all treatments that contained initial fertilizer applications. Treatments that supplied initial KV alone exhibited the lowest shoot weights (0.73–0.79 g), which were statistically similar to the untreated check (0.68 g). At 6 WAS, the greatest root weight was observed in response to sequential fertilizer applications (5.32 g), fertilizer + KV at seeding + KV 3 WAS (3.84 g), and single applications of fertilizer (3.58 g; Table 4). Treatments containing KV alone resulted in the lowest root weights (2.16–2.45 g), which were less than the untreated check (2.57 g) 6 WAS. Sequential applications of fertilizer resulted in the greatest shoot weight (9.15 g) 6 WAS followed by fertilizer + KV at seeding + KV 3 WAS (7.68 g) and a single application of fertilizer at seeding (6.27 g). All other treatments resulted in shoot weights ≤ 4.95 g and were statistically similar to the untreated check (4.82 g) 6 WAS.

The greatest root weight (0.57–0.71 g) 3 WAS in experimental run 2 was observed in response to treatments in which fertilizer was applied at seeding (Table 4). Treatments that supplied KV initially resulted in root weights of 0.24 to 0.36 g and were statistically comparable to the untreated check (0.30 g) 3 WAS. A similar trend was observed with respect to shoot weight 3 WAS. Single and sequential applications of fertilizer resulted in the greatest root weights (3.15–3.83 g) 6 WAS, whereas the other two treatments containing fertilizer resulted in similar root weights (2.44–2.57 g) as the untreated check (1.99 g; Table 4). The least root weights (1.59–1.64 g) were produced 6 WAS in response to treatments supplying KV only. Single and sequential applications of fertilizer resulted in the greatest shoot weights (7.71–8.21 g) 6 WAS; however, fertilizer at seeding + KV 3 WAS produced a comparable root weight (5.42 g). All other treatments resulted in statistically similar or lower root weights 6 WAS as the untreated check.

Treatments containing fertilizer consistently had greater NDVI, TC, and TQ than treatments that contained KV only. Establishment of bermudagrass plots in response to treatments containing fertilizer were nearly at full cover at the conclusion of the study (8 WAS), whereas treatments containing KV only were ≤ 70% cover and similar to the untreated check. However, the greatest ΔCE was often observed in the field in response to treatments that supplied KV 3 WAS. In the greenhouse, the greatest root and shoot weights were typically observed in response to treatments containing fertilizer, whereas KV-alone treatments resulted in root and shoot weights either similar to or less than the untreated check. Similarly, Peacock and Daniel (1992) observed a significantly greater hybrid bermudagrass growth rate in the greenhouse in response to urea fertilizer (3187 $mg \cdot m^{-2} \cdot d^{-1}$) than inoculation with *Bacillus* spp. (1903 $mg \cdot m^{-2} \cdot d^{-1}$) 17 d after treatment. Only a 17% increase in bermudagrass shoot growth with no increase in root growth was observed by Baltensperger et al. (1978) in response to inoculation with *Azospirillum* and *Azotobacter* spp. under low N fertility in the greenhouse. Contrarily, Coy et al. (2014) reported enhancements in root and shoot growth of 'Tifway 419' hybrid bermudagrass after inoculation with blends of *Bacillus*, *Paenibacillus*, and *Brevibacillus* spp. Although bermudagrass did not receive fertility during the trial, Coy et al. (2014) supplied bermudagrass with fertility for 3 consecutive weeks before trial initiation. Furthermore, mature hybrid bermudagrass transplants used by Coy et al.

Table 4. Root and shoot weight of seeded 'Sahara II' common bermudagrass [*Cynodon dactylon* (L.) Pers.] in response to fertility and microbial inoculants applied in the greenhouse during Spring 2021 in Athens, GA.

	Experimental run 1				Experimental run 2			
	Harvest,[y] 3 WAS		Harvest, 6 WAS		Harvest, 3 WAS		Harvest, 6 WAS	
Treatment[z]	Root wt (g)	Shoot wt (g)	Root wt (g)	Shoot wt (g)	Root wt (g)	Shoot wt (g)	Root wt (g)	Shoot wt (g)
Untreated check	0.28 d[x]	0.68 b	2.57 b	4.82 bc	0.30 bc	0.64 b	1.99 bc	4.57 bc
F at seeding	0.43 a–c	1.17 a	3.58 ab	6.27 a–c	0.57 ab	1.18 a	3.15 ab	7.71 ab
F at seeding + 3 WAS	0.49 ab	1.45 a	5.32 a	9.15 a	0.69 a	1.2 a	3.83 a	8.21 a
KV at seeding	0.32 cd	0.73 b	2.45 b	3.85 c	0.24 c	0.56 b	1.64 c	3.7 c
KV at seeding + 3 WAS	0.33 cd	0.79 b	2.16 b	4.95 bc	0.26 c	0.51 b	1.59 c	3.61 c
F at seeding + KV 3 WAS	0.56 a	1.45 a	3.36 b	4.84 bc	0.67 a	1.34 a	2.44 bc	5.42 a–c
F + KV at seeding + KV 3 WAS	0.41 b–d	1.15 a	3.84 a	7.68 ab	0.71 a	1.45 a	2.57 bc	4.89 bc
$LSD_{(0.05)}$	0.13	0.33	1.88	3.05	0.28	0.39	1.19	3.03

[z]Fertilizer was applied by hand using a shaker jar at a rate of 24 $kg \cdot ha^{-1}$ nitrogen. A stock solution of the microbial inoculant was prepared with distilled water and applied with a 50-mL syringe at an inoculation rate of 1.87 $L \cdot ha^{-1}$ (4×10^8 CFU/mL) and a carrier volume of 3000 $L \cdot ha^{-1}$.

[y]Destructive harvests were conducted 3 and 6 WAS. Roots and shoots were separated from each other, washed of all soil, dried in an oven for 48 h at 110 °C, and weighed to determine root and shoot biomass.

[x]Means within a column followed by the same lowercase letter are not significantly different at $P \leq 0.05$ according to Fisher's protected least significant difference test.

F = fertilizer ($5N–5P_2O_5–5K_2O$); KV, microbial inoculant containing *Klebsiella variicola*; $LSD_{(0.05)}$ = least significant difference at $P \leq 0.05$; WAS = weeks after seeding.

(2014) may have been more conducive to colonization than the common bermudagrass established from seed in our research. Increases in root and shoot weight of other C-4 grasses [pearl millet, *Pennisetum glaucum* (L.) R. Br.; foxtail millet, *Setaria italica* (L.) P. Beauv.] in response to *Azospirillum* spp. have been documented; however, all these grasses exhibit annual growth habits (Di Ciocco and Cáceres, 1994; Mane et al., 2000; Rafi et al., 2012).

Most turfgrass research documenting benefits from use of microbial inoculants has examined the response of cool-season grasses planted in sterile media and grown in controlled environments (Acikgoz et al., 2016; DeLuca et al., 2020). Therefore, microbial inoculants are often evaluated without competition from native microorganisms or under local environmental conditions. Coy et al. (2019) documented nitrogenase activity and microbial persistence in root and shoot tissue of common bermudagrass after field inoculation with *Bacillus* spp. However, bacterial strains used in their research were collected previously from native populations and isolated by Auburn University's Department of Entomology and Plant Pathology, ensuring some adaptation to indigenous environments and competition with endemic microorganisms. The *K. variicola* bacterial inoculant used in our research has been gene-edited to continue N fixation even in the presence of high soil N (Bloch et al., 2020). Furthermore, it has also been gene-edited to decrease glutamine synthase production and activity, thus allowing bacteria to fix N in the presence of high soil inorganic N. Although these traits confer advantages, bacteria contained within this inoculant were isolated originally from the mucilage of aerial roots of an indigenous landrace of maize grown in Mexico (Van Deynze et al., 2018). Therefore, microorganisms adapted to our soil and environmental conditions may pose enough competition to reduce potential for colonization and N fixation from our *K. variicola* bacteria. In addition, although properly stored, a reduction in cfu's of the liquid *K. variicola* formulation used in our research may have occurred between manufacturing and application. Freeze-dried materials that are rehydrated before treatment do not experience these potential shortcomings.

Intensely managed turfgrass requires large amounts of management inputs (fertilizer, irrigation, pesticides) and cultural practices (aerification, top-dressing) to sustain adequate growth and functionality. However, these same inputs often influence microbial activity, persistence, and species diversity through the alteration of the physical and chemical characteristics of the soil. In addition, turfgrass species may play a critical role in the potential for symbiotic relationships between plant roots and soil microorganisms. Research conducted by our group examined only one or two microbial inoculation events and monitored bermudagrass response over a short period of time. Coy et al. (2014) reported enhancement in root and shoot growth in response to weekly microbial inoculation over a 5-week period. Furthermore, documented growth enhancement of agronomic crops observed in response to *K. variicola* applications may be a result of reduced microbial competition from recurrent tillage events not conducted in turfgrass environments. Future research should examine the impact of more frequent and numerous inoculation events on turfgrass performance over multiple growing seasons to determine the feasibility of colonization and potential adoption of more sustainable turfgrass N management.

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