BACTERIAL POPULATIONS AND DIVERSITY WITHIN NEW USGA PUTTING GREENS

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Project conducted in collaboration
with M. Elliott (Univ. Florida) and H. skipper (Clemson Univ.)

EXECUTIVE SUMMARY

Conducted in cooperation with Clemson University (H.D. Skipper) and the University of Florida (M.L. Elliott) this study evaluates bacterial species and their population fluxes in the soil and rhizosphere during the establishment and maintenance of putting greens. Treatments in this study include grass type (bent or bermuda), organic construction material (reed peat moss vs. sphagnum), fumigants (methyl bromide, metam sodium or dazomet) and N fertility regimes (1/10 or 1/5 lb N/1,000 ft^2/week). At Auburn University treatments are N rate and construction materials (100% sand or 80/20 sand/peat). Sixteen containerized greens were constructed at the Auburn University Turfgrass Research Unit, four replications of each fertility/soil mix combination. Each green is 1 m long and 0.5 m wide, and each drains to an individual leachate collection chamber. Total leachate is collected from each green each week (or as-needed), and a subsample collected for NO_3-N and NH_4-N analysis. In February, May, August and November of each year root and soil samples (0-4 in. depth) are collected from each green. An additional soil sample is also collected for NO_3-N and NH_4-N analysis. Root samples are shipped to the Univ. of Florida where they are subjected to dilution plating and identification. Selected isolates are returned to Auburn Univ., where identification at the species level is conducted via GC FAME analysis. Results of 3 years of leachate collection have revealed that, once grow-in and application of higher rates of N was completed (a rate of 1 or 2 lb N/1,000 ft^2/month) was used for the first three months of the study) little NO_3-N or NH_4-N leaches through the rooting profile of the putting greens. During the past two years no more than 2 ug NO_3-N/ml of leachate has been collected at any one sampling, indicating the frequent application of low rates of N has maintained quality turf and reduced NO_3-N leaching. Preliminary evaluation of 8 dilution platings (from Univ. FL data) indicates that populations of selected bacterial species were affected by both N rate and putting green mix. There were no strong trends in bacterial populations over time.

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INTRODUCTION

Standards for building putting greens have been developed by the USGA such that greens throughout the country will have similar physical, and to a lesser extent, similar chemical characteristics at the time they are constructed and planted. However, there is limited research which examines microbial characteristics associated with the construction materials of putting greens or examined the flux in microbial populations and diversity on turfgrass roots after the greens have been planted. This USGA-funded project examines the interrelationships between microorganisims as influenced by their biotic (living organisms) and abiotic (construction materials) environment in new putting greens. Conducted as a cooperative effort with Clemson University and the University of Florida this project examines the effect of a variety of factors on microbial populations.

OBJECTIVES

The overall objective is to develop baseline data concerning bacterial composition (population and diversity) of new USGA putting greens, both during and after construction. Specific objectives include:

1. Determine bacterial populations associated with putting green root-zone mix materials (AL, SC and FL)

2. Determine bacterial populations of the root-zone mixes before and after fumigation (FL)

3. Compare bacterial populations associated with two different turfgrass species (AL - bentgrass, SC & FL - bermudagrass)

4. Compare thatch development, rooting and bacterial population of bentgrass in relation to N rate and putting green mix (AL)

5. Document rhizosphere bacterial population dynamics on bentgrass and bermudagrass putting greens over a four year period (AL, SC and FL)

METHODS AND MATERIALS - Auburn University

Sixteen containerized greens were built at the Auburn University Turfgrass Research Center, each designed to drain completely into an individual collection chamber. Greens are 1 m long and 0.5 m wide, and were sodded with washed bentgrass (cv 'Crenshaw') sod in January 1997. Treatments consisted of four replications of two greens mixes (sand or an 80/20 sand/peat mix) and two rates of N fertilizer (1/5 or 1/10 lb N/1,000 ft^2/week). Nitrogen is applied as a solution via a CO_2 backpack sprayer. Additional fertility (P, K, Ca, Mg, micros and lime) is surface applied as a granular material on an as-needed basis. All plots are irrigated uniformly and pesticide and fungicide are also applied uniformly to all plots. Greens are mowed 6 days of 7 at a 5/32 in. mowing height. Topdressing and core aerification are applied on an as-needed basis, with topdressing applied at least monthly and aerification (core) performed at least three times per year.

Leachate from each green is collected whenever necessary, and total volume of leachate is measured. A subsample is collected, filtered and frozen until analyses for NO_3-N and NH_4-N. Four times per year (February, May, August, November) 0-4 in. soil and root samples are collected from each plot. These are shipped to the University of Florida, where dilution plating is performed to identify general families of bacteria. Once isolates have been identified they are returned to Auburn University, where species identification is performed via GC FAME techniques. Additional data collection from the Auburn University plots includes monthly VAM (vesicular arbuscular mychorrizae) counts and quarterly soil NO_3-N and NH_4-N analyses.

RESULTS AND DISCUSSION

Nine quarterly samplings of the plots have been completed to date. Results from the dilution plating are presented in this 1999 report (Tables 1-9, attached). These dilution platings were used to select isolates for GC FAME analysis, data which has not yet been analyzed at this date.

Dilution plating data from the University of Florida revealed that there was rarely a significant putting green mix x N rate interaction at any sampling. However, the main effects of greens mix or N rate often did produce significant differences in bacteria population. Specifically, the presence of OM in the greens mix sometimes produced higher numbers of Fluorescent pseudomonads (May 1997 and May 1998) and a greater number of total bacteria (May 1997, Nov 1997, May 1998 and Sept 1998). Populations of gram-positive bacteria were never affected by greens mix or N rate. This was the only plating that was never affected by treatment.

Populations of plated bacteria were typically constant over time, and did not increase or decrease dramatically with time. The only plating that did show change was actinomycetes, where none were counted at the first sampling (May 1997). However, after this first sampling numbers of actinomycetes increased to an average of 6.2 $\log_{10}$ cfu/gram, remaining at or near this level for the remainder of the samplings.

These quarterly samplings represent almost three years of sampling, and data from this part of the study is currently being prepared for submission to *Phytopathology* as a refereed journal article. GC FAME data collected from this study will be submitted as a separate scientific article. Collected NO_3-N and NH_4-N leaching data will be submitted as note to the Soil Science Society America Journal at the end of four years of data collection.

Table 1. Effect of N rate and greens mix on selected microbial populations, May 1997.

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	log$_{10}$ cfu/gram		
Sand	6.21 b	5.27 a	7.39 a
USGA	6.44 a	5.16 a	7.47 a
1/10 lb N/1,000 ft^2/week	6.18 b	5.01 a	7.38 a
1/5 lb N/1,000 ft^2/week	6.47 a	5.42 a	7.48 a
Mix x N Rate interaction (P-Value)	0.889	0.526	0.774

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	log$_{10}$ cfu/gram			
Sand	6.07 b	0	7.66 b	5.52 a
USGA	6.44 a	0	8.34 a	5.57 a
1/10 lb N/1,000 ft^2/week	6.03 b	0	7.99 a	5.35 a
1/5 lb N/1,000 ft^2/week	6.44 a	0	8.00 a	5.74 a
Mix x N Rate interaction	0.007	0	0.994	0.180

Table 2. Effect of N rate and greens mix on selected microbial populations, August 1997.

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	---------- log_{10} cfu/gram ----------		
Sand	5.16 a	3.64 a	7.06 a
USGA	5.45 a	3.46 a	7.18 a
1/10 lb N/1,000 ft^2/week	5.26 a	3.69 a	7.10 a
1/5 lb N/1,000 ft^2/week	5.35 a	3.41 a	7.14 a
Mix x N Rate interaction (P-Value)	0.251	0.553	0.069

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	---------- log_{10} cfu/gram ----------			
Sand	4.67 a	6.15 a	8.08 a	5.80 a
USGA	5.50 a	6.22 a	8.19 a	5.84 a
1/10 lb N/1,000 ft^2/week	5.18 a	6.20 a	8.11 a	5.63 b
1/5 lb N/1,000 ft^2/week	4.99 a	6.17 a	8.16 a	6.01 a
Mix x N Rate interaction	0.415	0.284	0.093	0.473

Table 3. Effect of N rate and greens mix on selected microbial populations, November 1997.

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	---------- log_{10} cfu/gram ----------		
Sand	5.07 a	3.50 a	7.39 a
USGA	5.43 a	4.07 a	7.40 a
1/10 lb N/1,000 ft^2/week	5.19 a	3.76 a	7.18 b
1/5 lb N/1,000 ft^2/week	5.31 a	3.81 a	7.61 a
Mix x N Rate interaction (P-Value)	0.989	0.542	0.307

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	---------- log_{10} cfu/gram ----------			
Sand	4.05 a	6.33 a	8.07 b	6.39 a
USGA	4.21 a	6.64 a	8.29 a	6.49 a
1/10 lb N/1,000 ft^2/week	4.21 a	6.58 a	8.09 b	6.03 b
1/5 lb N/1,000 ft^2/week	4.04 a	6.40 a	8.26 a	6.85 a
Mix x N Rate interaction	0.005	0.199	0.640	0.866

Table 4. Effect of N rate and greens mix on selected microbial populations, February 1998.

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	---- $\log_{10}$ cfu/gram ----		
Sand	6.28 a	2.83 a	7.64 a
USGA	6.08 a	3.63 a	7.51 a
1/10 lb N/1,000 ft^2/week	6.21 a	3.35 a	7.35 b
1/5 lb N/1,000 ft^2/week	6.14 a	3.11 a	7.80 a
Mix x N Rate interaction (P-Value)	0.692	0.611	0.195

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	---- $\log_{10}$ cfu/gram ----			
Sand	2.90 a	6.22 a	8.26 a	6.15 b
USGA	2.62 a	6.12 a	8.37 a	6.58 a
1/10 lb N/1,000 ft^2/week	2.98 a	6.10 a	8.20 b	5.91 b
1/5 lb N/1,000 ft^2/week	2.53 a	6.23 a	8.43 a	6.81 a
Mix x N Rate interaction	0.740	0.215	0.343	0.513

Table 5. Effect of N rate and greens mix on selected microbial populations,May 1998.

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	log$_{10}$ cfu/gram		
Sand	6.24 b	3.25 a	7.72 a
USGA	6.38 a	3.71 a	7.60 b
1/10 lb N/1,000 ft^2/week	6.28 a	3.72 a	7.46 b
1/5 lb N/1,000 ft^2/week	6.34 a	3.23 a	7.86 a
Mix x N Rate interaction (P-Value)	0.408	0.946	0.0001

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	log$_{10}$ cfu/gram			
Sand	4.18 a	6.21 a	8.17 b	5.93 a
USGA	4.38 a	6.35 a	8.25 a	6.20 a
1/10 lb N/1,000 ft^2/week	4.15 a	6.17 a	8.16 b	5.64 b
1/5 lb N/1,000 ft^2/week	4.41 a	6.39 a	8.27 a	6.49 a
Mix x N Rate interaction	0.250	0.862	0.012	0.665

Table 6. Effect of N rate and greens mix on selected microbial populations, September 1998.

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	log_{10} cfu/gram		
Sand	6.34 a	3.18 a	7.19 a
USGA	6.24 a	3.22 a	7.26 a
1/10 lb N/1,000 ft^2/week	6.41 a	3.16 a	7.13 b
1/5 lb N/1,000 ft^2/week	6.17 a	3.25 a	7.32 a
Mix x N Rate interaction (P-Value)	0.788	0.227	0.840

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	log_{10} cfu/gram			
Sand	4.61 a	6.54 a	8.08 b	6.04 a
USGA	4.58 a	6.41 a	8.23 a	6.19 a
1/10 lb N/1,000 ft^2/week	4.17 b	6.39 a	8.01 b	5.85 b
1/5 lb N/1,000 ft^2/week	5.01 a	6.56 a	8.29 a	6.38 a
Mix x N Rate interaction	0.112	0.015	0.616	0.488

Table 7. Effect of N rate and greens mix on selected microbial populations,November 1998.

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	---------- $\log_{10}$ cfu/gram ----------		
Sand	5.97 a	5.05 a	6.65 a
USGA	5.97 a	5.40 a	6.68 a
1/10 lb N/1,000 ft^2/week	6.05 a	5.13 a	6.67 a
1/5 lb N/1,000 ft^2/week	5.89 a	5.32 a	6.66 a
Mix x N Rate interaction (P-Value)	0.507	0.166	0.367

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	---------- $\log_{10}$ cfu/gram ----------			
Sand	4.38 a	6.66 a	7.70 a	6.11 a
USGA	4.23 a	6.61 a	7.50 a	6.18 a
1/10 lb N/1,000 ft^2/week	4.23 a	6.67 a	7.66 a	6.00 a
1/5 lb N/1,000 ft^2/week	4.38 a	6.60 a	7.54 a	6.28 a
Mix x N Rate interaction	0.022	0.676	0.729	0.643

Table 8. Effect of N rate and greens mix on selected microbial populations,February 1999

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	------ log_{10} cfu/gram ------		
Sand	3.71 a	3.70 a	4.17 a
USGA	4.40 a	3.82 a	4.83 a
1/10 lb N/1,000 ft^2/week	3.79 a	3.66 a	4.16 a
1/5 lb N/1,000 ft^2/week	4.33 a	3.86 a	4.84 a
Mix x N Rate interaction (P-Value)	0.327	0.198	0.509

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	------ log_{10} cfu/gram ------			
Sand	1.84 b	3.85 a	4.99 a	3.37 a
USGA	2.62 a	4.47 a	5.85 a	4.01 a
1/10 lb N/1,000 ft^2/week	1.90 b	3.82 a	5.00 a	3.31 a
1/5 lb N/1,000 ft^2/week	2.56 a	4.49 a	5.84 a	4.07 a
Mix x N Rate interaction	0.005	0.579	0.349	0.340

Table 9. Effect of N rate and greens mix on selected microbial populations, May 1999

Mix/N Rate	Fluorescent pseudomonads	Gram-positive bacteria	Gram-negative bacteria
	log$_{10}$ cfu/gram		
Sand	5.00 a	4.96 a	7.57 a
USGA	6.56 a	5.19 a	7.70 a
1/10 lb N/1,000 ft^2/week	4.93 a	5.04 a	7.53 b
1/5 lb N/1,000 ft^2/week	6.62 a	5.11 a	7.74 a
Mix x N Rate interaction (P-Value)	0.143	0.438	0.763

	Maltophilia-like bacteria	Actinomycetes	Total bacteria	Heat-tolerant bacteria
	log$_{10}$ cfu/gram			
Sand	4.88 a	6.11 b	8.13 a	5.94 a
USGA	4.98 a	6.46 a	8.20 a	6.00 a
1/10 lb N/1,000 ft^2/week	4.81 a	6.18 a	8.14 a	5.74 b
1/5 lb N/1,000 ft^2/week	5.05 a	6.39 a	8.21 a	6.20 a
Mix x N Rate interaction	0.668	0.576	0.584	0.633