Microbial Populations and Diversity in Bentgrass Putting Greens as Affected by N Rate and Root-Zone Mix

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EXECUTIVE SUMMARY

Microbial diversity in constructed and fumigated golf course putting greens is a topic that has not been widely explored. The objective of this project was to evaluate the microbial diversity of bentgrass (Agrostris palustris 'Crenshaw') greens over time as affected by root-zone mix and N rate. Two root-zone mixes (80/20% sand/peat and 100% sand) and two N rates (1.0 and 0.5 g N m-2 week-1) were evaluated, with four reps of each root-zone/N rate treatment built in individual separated mini greens (1 m x ½ m). Quarterly (Feb., May, Aug., Nov.) 0-7.6 cm root samples were removed from each mini-green. Sampling was performed for three years. Standard dilution plating techniques were used to evaluate populations of fluorescent pseudomonads, gram-positive bacteria, gram-negative bacteria, S. maltophilia-like bacteria, actinomycetes and heat-tolerant bacteria. There was rarely a significant N rate x root-zone mix interaction, although main effects of N rate and root-zone mix did affect microbial diversity at some samplings. If differences did exist, bacterial counts were higher in the sand/peat root zone mix or in the treatments receiving the higher rate of N. There were no obvious population trends in any microbial population over the three year sampling period. Populations of total bacteria ranged from a low of 5.4 to a high of 8.3 $\log_{10}$ cfu/gram throughout the two-year sampling period.

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INTRODUCTION

Standards for building 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, little research has examined the microbial characteristics associated with newly constructed putting greens and the flux in microbial population and diversity on turfgrass roots, after the greens have been planted.

Research on microbial populations associated with turfgrass has been limited. Little or no attempt has been made to separate microbial populations into more than one or two major subgroups. Furthermore, microbial populations have not been determined for the rhizosphere but rather bulk soil, a composite of bulk and rhizosphere soil, or thatch.

OBJECTIVES

The objectives of this project were to evaluate microbial numbers and diversity in bentgrass putting greens over time as affected by root-zone mix and N rate.

Putting Green Construction and Maintenance

In the fall of 1997 miniature putting greens were constructed at the Auburn University Turfgrass Research Unit, Auburn, AL. Each mini-green was 1 m x 1/2 m x 0.5 m and sealed so that drainage was collected below each minigreen. Sixteen minigreens were constructed and buried so that the putting surface of each green was at ground level. Greens mix treatments were pure sand and 80/20 sand/peat (Reed Sedge Peat). The sand/peat mix fit all USGA recommended guidelines for greens mix. On March 26 1997 washed bentgrass sod (*Agrostis palustris* Crenshaw') was installed in every minigreen.

Treatments for the study were arranged as a 2 x 2 factorial of greens mix (sand or sand/peat) and N rate (0.5 or 1 .0 g $N/m^{-2}/week^{-1}$), with 4 replications of each greens mix/N rate combination. Nitrogen was applied weekly using a pressurized CO_2 backpack sprayer and a soluble fertilizer (20-5-10, N-P_2O_5-K_2O) source, with additional urea added for the higher N rate. All additional maintenance (fungicides, herbicides, insecticides) were applied on an as-needed and as-recommended basis. Plots were core aerified (6 mm hollow tines to a depth of 10 cm) approximately every three months, verticut twice a year and topdressed twice a month. Water was applied to supply 2.5 cm water $week^{-1}$. Plots were maintained by daily mowing at a 4 mm height.

RESULTS AND DISCUSSION

Analysis of variance of the data was conducted at each sampling date for each measured microbial population. There were few significant N rate x greens mix interactions, indicating that the main effects of greens mix and N rate affected microbial populations independently (Table 1). The most consistent and significant greens mix x N rate interaction occurred for *S. maltophilia* -like bacteria, with significant interactions occurring at the May 97, Nov., 97, Nov., 98 and Feb. 99 samplings (Table 2). In that case the interaction usually occurred because there were lower populations of *S. maltophilia* at low rates of N in the sand greens mix, while populations in the peat mix were inconsistently affected by N rate (Table 2).

Effect of Greens Mix on Microbial Populations

Populations of gram-positive bacteria were never affected by type of greens mix, with an average population of 4.06 $\log_{10}$ cfu/gram of root tissue over the two years of sampling. Other microbial populations that were largely unaffected by greens mix were gram-negative bacteria, actinomycetes (Figure 1, averaged over N rate) and heat-tolerant bacteria. Gram-negative bacteria had one sampling with significant differences due to greens mix - May 1998 populations in sand/peat were lower than in sand (7.60 versus 7.72 log10 cfu/gram). Heat-tolerant bacteria also had only one sampling where greens mix affected populations, and in this case there were more heat-tolerant bacteria (6.58 $\log_{10}$ cfu/gram) in the sand/peat mix than in sand (6.15 $\log_{10}$ cfu/gram).

The microbial population most affected by greens mix was total bacteria, with 4 of the 9 samplings showing differences (Figure 2, averaged over N rate). In all of those samplings there were more total bacteria in the sand/peat mix than in pure sand. Populations of fluorescent pseudomonads behaved similarily, and in the two samplings where greens mix did affect population of pseudomonads, it was greater in the sand/peat mix than sand (Figure 3, averaged over N rate).

Effect of N Rate on Microbial Populations

The only microbial populations which were unaffected by N rate were actinomycetes and gram-positive bacteria. These were the same populations which were largely unaffected by greens mix. Every other microbial population had at least one sampling that was affected by N rate (Figures 4 through 6, averaged over greens mix), and in every case the higher N rate always had higher populations of that microbial population.

CONCLUSIONS

1. Selected microbial populations of fluorescent pseudomonads, gram-negative bacteria, S. maltophilia-like bacteria, total bacteria and heat-tolerant bacteria were affected by N rate and greens mix. Except for *S. maltophilia*, if a significant difference occurred there were usually larger populations in the sand/peat mix than sand, and always larger populations in the higher N rate than lower.

2. Populations of gram-positive bacteria were unaffected by N rate or greens mix, and populations of actinomycetes were only affected at one sampling (May 1999, greens mix only).

3. The cause of the sharp drop in populations of all plated microbes in February 1999 is unknown. Other than that drop the flux of microbial populations across all samplings was consistent.

Table 1. Significance of N rate and greens mix on selected microbial populations from the root zone of bentgrass putting greens, May 1997 to May, 1999, Auburn, AL.

Factor	May 97	Aug 97	Nov 97	Feb 98	May 98	Sept 98	Nov 98	Feb 99	May 99
	Prob > F								
	Fluorescent pseudomonads								
N Rate	0.014	0.537	0.587	0.303	0.303	0.122	0.615	0.331	0.132
Greens Mix	0.047	0.088	0.142	0.734	0.040	0.459	0.983	0.223	0.157
N Rate x Mix	0.889	0.251	0.989	0.692	0.408	0.788	0.507	0.327	0.156
	Gram-positive bacteria								
N Rate	0.119	0.655	0.852	0.718	0.067	0.509	0.515	0.736	0.796
Greens Mix	0.639	0.765	0.054	0.268	0.080	0.787	0.250	0.828	0.439
N Rate x Mix	0.526	0.553	0.542	0.611	0.946	0.227	0.166	0.198	0.437
	Gram-negative bacteria								
N Rate	0.197	0.722	0.008	0.009	0.0001	0.037	0.963	0.317	0.011
Greens Mix	0.288	0.376	0.928	0.329	0.0001	0.349	0.878	0.327	0.052
N Rate x Mix	0.774	0.069	0.307	0.195	0.0001	0.840	0.367	0.509	0.763
	S. maltophilia-like bacteria								
N Rate	0.001	0.298	0.292	0.298	0.448	0.005	0.346	0.004	0.369
Greens Mix	0.003	0.002	0.312	0.504	0.566	0.900	0.361	0.001	0.715
N Rate x Mix	0.007	0.415	0.005	0.740	0.250	0.112	0.022	0.005	0.668
	Actinomycetes								
N Rate	NM	0.852	0.541	0.536	0.147	0.277	0.704	0.326	0.066
Greens Mix		0.678	0.317	0.625	0.336	0.365	0.796	0.228	0.009
N Rate x Mix		0.284	0.199	0.215	0.862	0.015	0.676	0.579	0.576
	Total bacteria								
N Rate	0.812	0.639	0.017	0.017	0.0007	0.002	0.518	0.252	0.542
Greens Mix	0.001	0.268	0.006	0.172	0.004	0.041	0.277	0.265	0.505
N Rate x Mix	0.994	0.093	0.640	0.343	0.012	0.616	0.729	0.349	0.584
	Heat-tolerant bacteria								
N Rate	0.073	0.008	0.002	0.0005	0.0003	0.012	0.156	0.136	0.031
Greens Mix	0.766	0.742	0.562	0.018	0.124	0.371	0.717	0.204	0.726
N Rate x Mix	0.180	0.473	0.866	0.513	0.665	0.488	0.643	0.340	0.633

Table 2. Interaction of N Rate and Greens Mix on populations of S. maltophilia-like bacteria at selected dates in a bentgrass putting green, Auburn, AL.

N Rate (g m^{-2})	Sand	80 Sand/ 20 Peat
	Date/Greens Mix Type	
	May 1997	
0.5	5.7 b	6.3 a
1.0	6.4 a	6.5 a
	Nov 1997	
0.5	3.8 b	4.6 a
1.0	4.3 a	3.8 b
	Nov 1998	
0.5	4.5 a	3.9 b
1.0	4.2 a	4.5 a
	Feb 1999	
0.5	1.2 b	2.6 a
1.0	2.5 a	2.6 a

Within each N rate, greens mix and date means followed by a different letter are significantly different from each other at a = 0.05. Values are $\log_{10}$ cfu/g dry root tissue

Figure 1. Flux of actinomycetes as affected by greens mix, 1997-1999

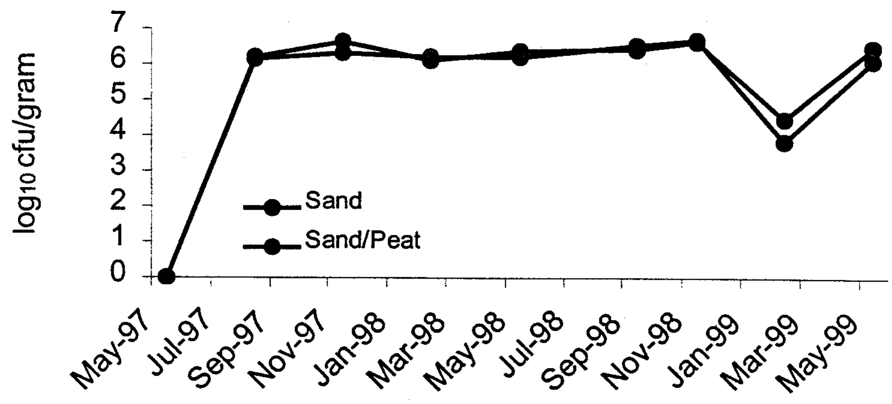

Figure 2. Flux of total bacteria as affected by greens mix, 1997-1999

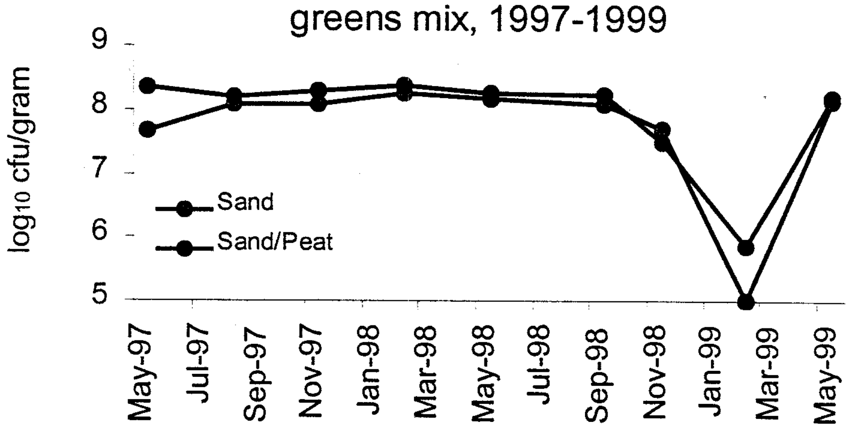

Figure 3. Flux of fluorescent psuedomonads as affected by greens mix, 1997-1999

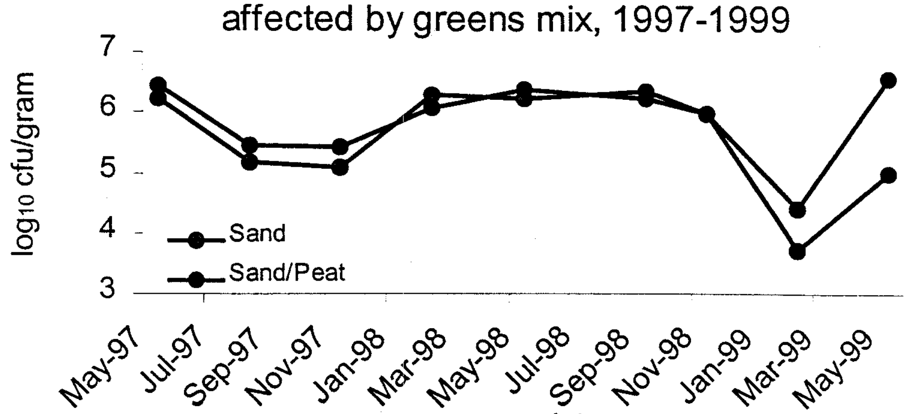

Figure 4. Flux of gram-negative bacteria as affected by N rate, 1997-1999

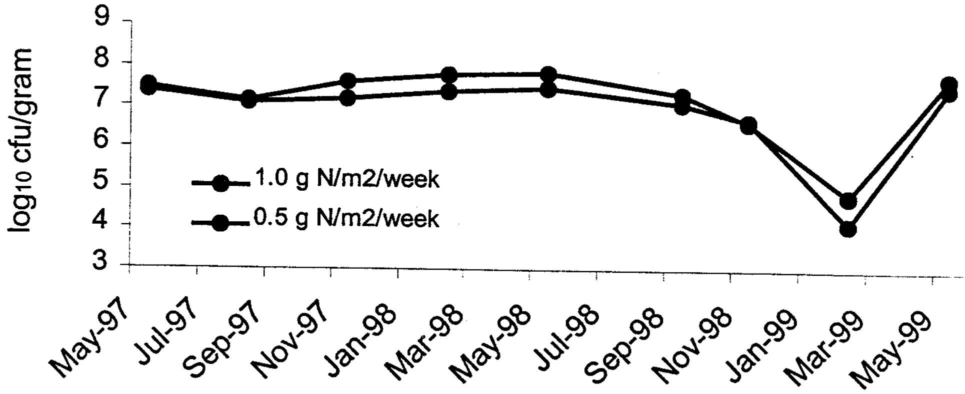

Figure 5. Flux of heat-tolerant bacteria as affected by N rate, 1997-1999

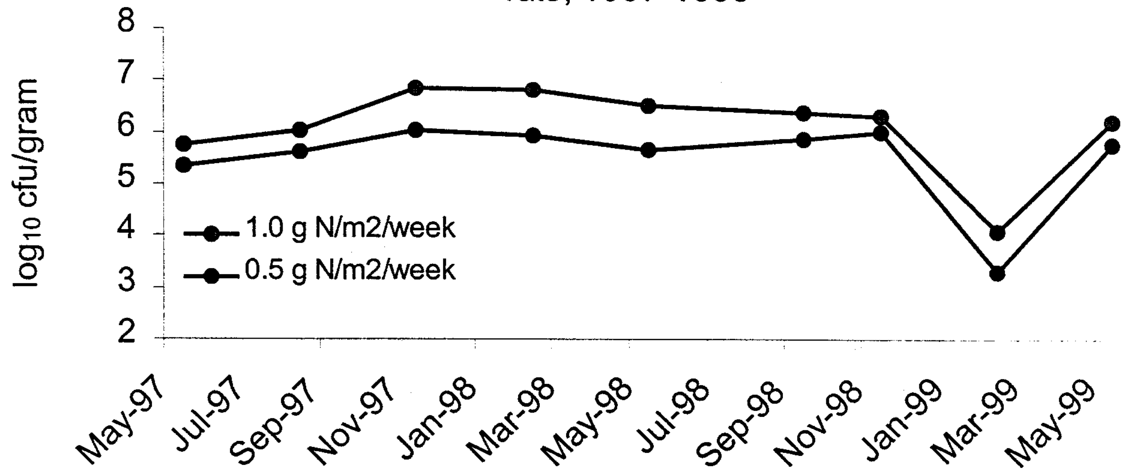

Figure 6. Flux of total bacteria as affected by N rate, 1997-1999