Turfgrass and Environmental Research Online

...Using Science to Benefit Golf

Miniature putting greens (shown above) were constructed at Auburn University to study the effects of rootzone mix and nitrogen fertility on the popoulation of bacteria surrounding creeping bentgrass roots in an effort to better understand the microbial ecology of putting green rootzones. Despite regular influxes of fungicides, pesticides, and fertilizers, seasonal flux of the bacterial populations evaluated in this study did not dramatically change over the three years of the study.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 290 projects at a cost of $25 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Microbe Populations in USGA Putting Greens

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SUMMARY

Microbial dynamics in USGA-type putting greens has become an area of intense interest in the past few years. Microbial inoculants or microbially-based growth promoters are often marketed to golf course superintendents, yet there is limited information about native bacterial populations in USGA-type putting greens. The objective of this research was to examine rhizosphere (around the roots) bacterial numbers in putting greens as affected by root-zone mix (100% sand or 80/20% sand/peat) and N rate (1/10 or 1/5 lb N 1,000 ft^{-2} $week^{-1}$).

- Miniature putting greens were constructed, with the four treatments randomized among the 16 miniature greens. Greens were sampled every three months, and roots and surrounding soil extracted for bacterial counts via dilution plating.
- Despite regular influxes of fungicides, pesticides and fertilizers, seasonal flux of the bacterial populations evaluated in this study did not dramatically change over the three years of the study.

The dynamics of microbes in turfgrass soils has become a topic of interest in both turfgrass research and turf-related commercial merchandising. From “microbial inoculants” to “compost teas”, supplements and additives that purport to change or affect the microbial pool have become big business. Such additives are often applied to turf without regard to existing bacterial and fungal populations in the turfgrass rhizosphere. This may occur because there is very little information available about bacterial populations in turfgrass soils and roots. It is easy to claim that a product or technique will alter the microbial pool when we know little about the populations of native microflora associated with turfgrass roots and soil.

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This lack of information is especially true on putting greens, a growing medium that is constructed from specific blends of sand, peat, or other organic amendment. Collecting information about the number and type of bacteria in a putting green is especially important as it is these intensively managed areas that are most likely to receive applications of microbial additives. The impact of turf management practices on bacterial flux is also an area of interest because inputs such as fertilizer, pesticides, or fumigation will affect the bacterial pool.

Bacterial flux has been studied in cropping systems, and seasonal changes in *Pseudomonas* from November to May has been documented in long-term (58-year-old) cropped plots (2). In other long-term (24-year-old) cropped plots, inputs of tillage, crop rotation, N fertilization, or time of sampling did not affect populations of actinomycetes or fungi. However, total bacterial counts were lower in treatments where N had been applied (11). This N-rate effect has been observed in other field crop research, as populations of *Pseudomonas spp.*, *Agrobacterium spp.*, and *Bacillus spp.* decreased in the rhizosphere of wheat, corn, and rye as N rate increased (9).

More closely related to turfgrasses were

Figure 1. Miniature putting greens were constructed at the Auburn University Turfgrass Research Unit, Auburn, AL

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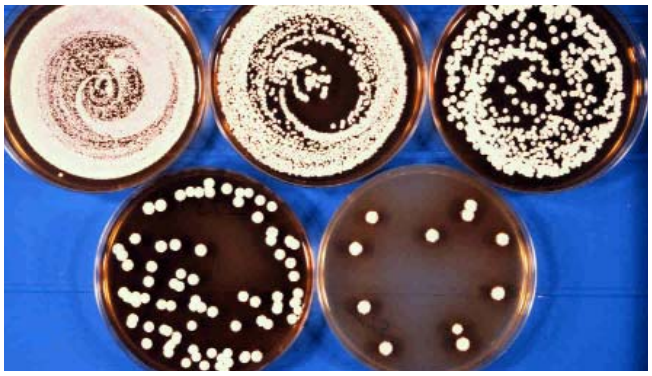

Figure 2. Aliquots of the dilutions were spread plated onto varying growth media from which bacterial counts were obtained.

studies that evaluated microbial population flux in grass crops which were usually cool-season grasses managed as a pasture. In a greenhouse study with perennial ryegrass, N and P fertilization had no effect on root surface microbial populations (12). Other studies have had similar results: when nitrate-N was applied at high rates to perennial ryegrass, tall fescue, annual bluegrass, or Kentucky bluegrass, rhizosphere bacteria were unaffected by N rate (8).

There have been a few studies which examined bacterial population flux in USGA-type sand/peat putting greens. These studies have examined the impact of fumigation and organic N source (4, 6) or microbe-enhanced organic N sources (10) on microbial populations. A third study also examined the impact of organic fertilizers on microbe populations, but that study was conducted on a native soil putting green, not a sand/peat mix (9). In general, addition of organic fertilizers did not increase soil or thatch bacterial populations, and there were rarely differences due to N source.

Lack of information about bacterial flux in USGA-type putting greens, coupled with the growing interest in this area, demonstrates the need to evaluate rhizosphere bacterial numbers as affected by rootzone mix and N rate. Rhizosphere (the area immediately around the roots) sampling, as opposed to bulk soil sampling, was also desired. Rhizosphere sampling provides an assessment of bacterial species immediately around the plant root, a population which is normally greater and more diverse than those obtained from bulk soil samples. Thus, the objective of this research was to examine rhizosphere bacterial numbers of diverse groups of bacteria in bentgrass putting greens as affected by rootzone mix and N rate, over a three-year period.

Materials and Methods

In February, 1997, miniature putting greens were constructed at the Auburn University Turfgrass Research Unit, Auburn, AL (Fig. 1). Each green was 1 x 0.5 x 0.5 m and sealed so that individual drainage from each green was collected below ground. Sixteen greens were constructed and buried so that the putting surface of each green was at ground level.

There were two rootzone mix treatments: 100% sand and a mix of 80% sand and 20% reed sedge peat (by volume). All sand and rootzone mix met USGA recommendations for porosity, saturated hydraulic conductivity, and particle size analysis. Rootzone mix treatments were placed into each mini-green on top of a gravel and intermediate layer. Greens were fumigated on March 7, 1997 using methyl bromide, and on March 26, 1997 washed creeping bentgrass sod (cv. Crenshaw) was planted on every green.

Treatments for the study were a 2 x 2 factorial of rootzone mix and N rate (0.5 or 1.0 g N m^{-2} $week^{-1}$ all year (1/10 or 1/5 lb N 1,000 ft^{-2} $week^{-1}$), with four replications of each rootzone mix x N rate combination. Soluble fertilizer (20-5-10) was used to apply the N rate treatments, with additional N (46-0-0) applied for the higher N rate treatment. Fungicides, insecticides, and herbicides were applied during the length of the study as required and as recommended.

Enumeration of Rhizosphere Bacterial Groups

Each of the 16 greens was sampled every three months from May, 1997 through February, 2000. Samples were obtained using a 1-cm diameter soil probe to a depth of 10 cm. Ten cores were removed from each green, a sterile razor blade was used to remove green material, and cores were collected in plastic bags. Samples

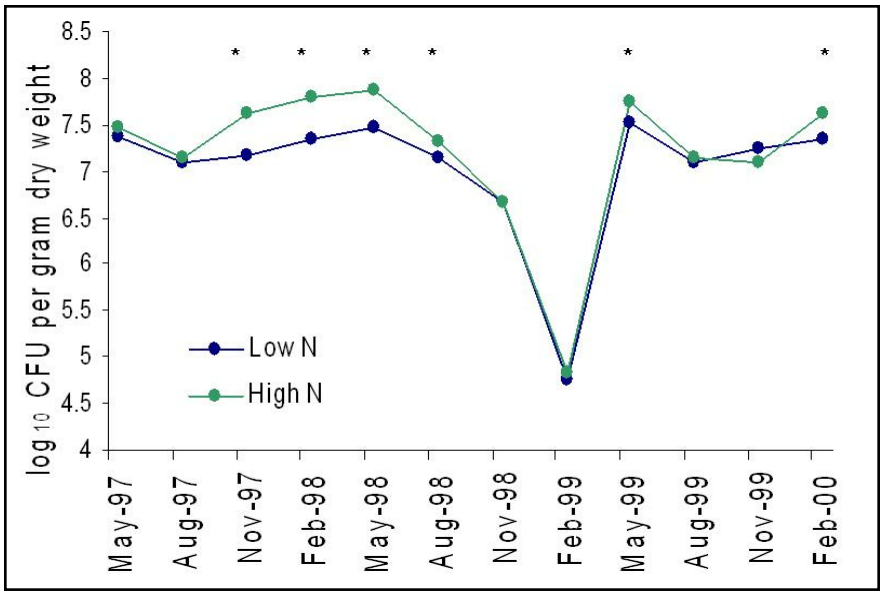

Figure 3. Numbers of gram-negative bacteria associated with the rhizosphere of 'Crenshaw' creeping bentgrass as affected by N rate, Auburn, AL. Values are means of eight replicates. An asterisk indicates a significant difference between N rates (P = 0.05).

were shipped overnight to the University of Florida where they were prepared for bacterial enumeration (3, 5).

To obtain bacterial counts, roots and adhering rhizosphere soil were shaken in a sterile flask with 95 mL of diluent, and the shaken solution was then subjected to a ten-fold dilution series. Aliquots of the dilutions were then spread plated onto varying growth media from which bacterial counts were obtained (Figure 2). Specific bacterial counts obtained were: 1) total aerobic bacteria, 2) flourescent pseudomonads, 3) *Stenotrophomonas maltophilia*-like bacteria , 4) actinomycetes, 5) gram-positive bacteria, 6) gram-negative bacteria, and, 7) heat-tolerant bacteria. Bacterial counts were performed at the appropriate time for each growth medium. All counts were reported as $\log_{10}$ CFU (colony forming units) per gram dry weight of root material. Details of exact media and techniques used for the dilution plating can be found in Elliott et al. (3).

Results and Discussion

There were a total of 12 dates when the bentgrass rhizosphere was sampled from May, 1997 until February, 2000 for the seven different bacterial populations. Out of these 84 samplings (12 dates of sampling x 7 bacterial groups) there were limited times that the rootzone mix x N rate interaction was significant. Numbers of heat-tolerant bacteria and total bacteria were never significantly affected by an interaction between N rate and rootzone mix. Populations of fluorescent pseudomonads, gram-positive bacteria, gram-negative bacteria, and actinomycetes had only one sampling date where the treatment interaction was significant, and this was not consistent across sampling dates.

Populations of *Stenotrophomonas mal-*

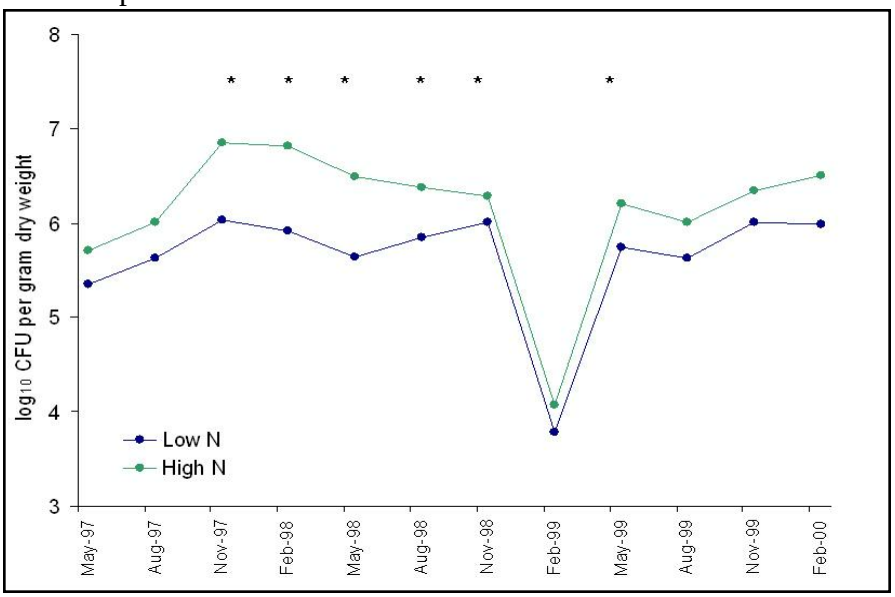

Figure 4. Numbers of heat-tolerant bacteria associated with the rhizosphere of 'Crenshaw' creeping bentgrass as affected by N rate, Auburn, AL. Values are means of eight replicates. An asterisk indicates a significant difference between N rates (P = 0.05).

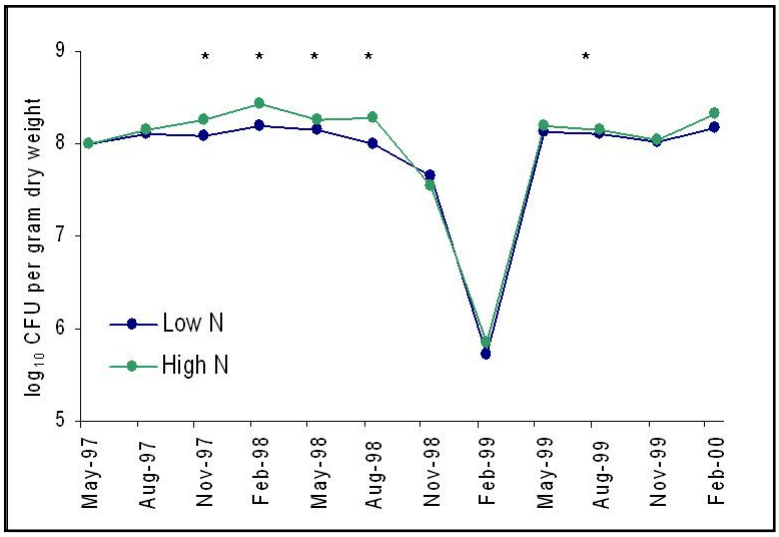

Figure 5. Numbers of total aerobic bacteria associated with the rhizosphere of Crenshaw creeping bentgrass as affected by N rate, Auburn, AL. Values are means of eight replicates. An asterisk indicates a significant difference between N rates (P = 0.05).

tophilia-like bacteria had four sampling dates at which the interaction of root-zone mix and N rate was significant. For this bacterial group, the interaction usually occurred because there were lower numbers of *S. maltophilia* in plots treated with the low N rate in the sand rootzone mix, while numbers in the sand-peat rootzone mix were unaffected by N rate. Limited significant interactions indicates that the main effects of rootzone mix and N rate largely affected bacterial population numbers independently.

For gram-negative bacteria, heat-tolerant bacteria and total aerobic bacteria, a higher N rate often increased the populations of those bacterial groups (Figures 3, 4 and 5). In six of 12 samplings, the higher N rate significantly increased numbers of gram-negative bacteria and heat-tolerant bacteria, and in five of 12 samplings the higher N rate increased numbers of total aerobic bacteria.

When viewed across sampling date (Figures 3, 4 and 5), there was a sharp drop in populations at the February, 1999 sampling date. This drop was significant for five of the seven bacterial groups, with only populations of gram-positive bacteria and *Stenotrophomonas maltophilia*-like bacteria not significantly decreasing. The reason for this sharp drop may be directly associated with a prolonged decrease in root biomass (Figure 6). There were no changes in pesticide spray schedules, nor were any fumigants or nematicides applied prior to this sampling. February mean air or soil temperatures did not significantly differ from 30-year averages. The prolonged decrease in root biomass would have eventually decreased root exudation and carbon availability to rhizosphere bacteria. This would have negatively impacted bacterial numbers, as carbon from the roots is considered the primary factor controlling microbial growth (7).

Rootzone mix also had significant effects on bacterial populations, with total aerobic bacteria (four dates) and *Stenotrophomonas maltophilia*-like bacteria (three dates) affected most often. In total, there were only 12 of 84 sampling events (a specific bacterial population counted at one date is an event) where rootzone mix significantly affected bacterial populations. At 10 of these 12 events, greatest populations were found in the sand-peat rootzone mix. The organic matter in the sand-peat mix may have provided a beneficial environment for increased populations of

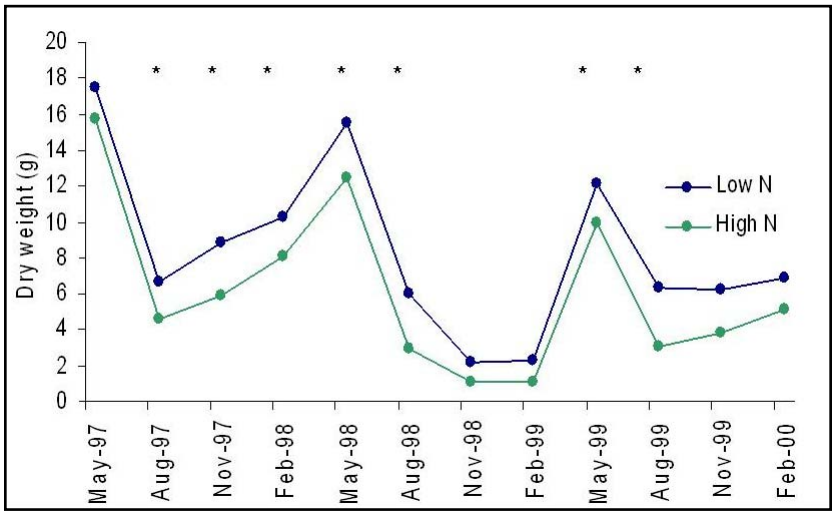

Figure 6. Root weights of Crenshaw creeping bentgrass as affected by nitrogen rate, Auburn, AL. Values are means of eight replicates. An asterisk indicates a significant difference between N rates (P = 0.05).

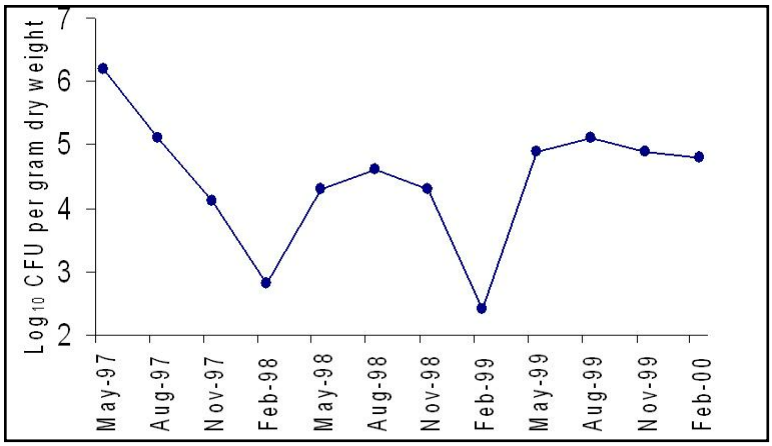

Figure 7. Effect of sampling date on populations of *S. maltophilia* - like bacteria from bentgrass putting greens at Auburn, AL. Values are means of 16 plots across all N levels and rootzone treatments, four replicate plots for each of four treatment combinations.

microflora (1).

Across all treatments, there was minimal fluctuation in populations of gram-negative bacteria, actinomycetes, heat-tolerant and total aerobic bacteria. Since these bacterial populations were determined from roots and rhizosphere soil, the numbers can be expressed per gram of root weight (as in Figures 3, 4 and 5). When February, 1999 data are eliminated, the greatest difference in sample populations from each date for these four bacterial groups is less than 1 log unit. Of all the bacterial populations studied, *Stenotrophomonas maltophilia*-like bacteria had the greatest population flux. Populations of that group of bacteria followed total roots weights closely (Figure 7). Differences in populations of *Stenotrophomonas maltophilia*-like bacteria varied by up to 3.4 log units.

Relative uniformity in soil or rhizosphere bacterial populations has been observed in previous studies on bermudagrass and bentgrass putting greens (4, 10). Despite regular influxes of fungicides, pesticides, and fertilizers, seasonal flux of the bacterial populations evaluated in this study did not dramatically change over the three years of the study. The fact that the populations evaluated were largely stable over time and only minimally affected by N rate and rootzone mix, provides a starting point for information about the robust nature of the bacterial pool in putting greens. Much more research in this area is needed, including the evaluation of alternative techniques for bacterial enumeration and population diversity. Additionally, the addition of bacterial or microbial inoculants and their effect on native microflora is another area requiring continued research.

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