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Biostimulant Influences on Turfgrass Microbial Communities and Creeping Bentgrass Putting Green Quality

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Abstract. **Immature sand matrix golf putting greens are considered to be inhospitable environments for microorganisms as compared to native soils. Subsequently, turfgrass quality may suffer in the absence of beneficial microbe–plant interactions. The turfgrass industry has responded by marketing a wide array of biostimulant products that claim to improve putting green quality through influences on soil microbial activity. A field study was conducted to determine what influences five commercial biostimulants have on the root-zone microbial community and creeping bentgrass (*Agrostis stolonifera* L.) quality. A three year old U.S. Golf Association (USGA) specification sand-based putting green (e.g., 80% sand: 20% peat humus by volume) was the test site. Commercially available biostimulants and fertilizer were applied biweekly from May until August 2000. The soil microbial community was characterized using soil enzymes and substrate utilization profiles. Turfgrass quality was determined visually by evaluating color, percentage of localized dry spot (LDS), and overall uniformity. Nutrient uptake levels were monitored to ascertain if increases in quality related to plant health. Visual quality of the putting green was significantly improved ($p < 0.05$) by the commercial biostimulants. The positive response to biostimulants was not of a nutritional origin. The biostimulants did not effectively alter the putting green microbial community in terms of enzyme activity or substrate utilization. However, a seasonal decline was detected in cellulase activity, which prevailed over any treatment effect, suggesting the root-zone microbial community responded to summer decline of bentgrass roots and concomitant decreases in quantities of root exudates. Visual improvements in putting green quality during the period of summer stress were primarily associated with the incidence of LDS. Visual LDS ratings were significantly reduced (less LDS) by applications of the biostimulants on each observation date ($p < 0.05$) and over the entire course of the experiment ($p < 0.10$). Surfactant properties of the biostimulants therefore appeared to play a major role in the improvements in putting green quality. This does not negate the fact that the seaweed extracts and humic acids in the biostimulants may have improved the heat and moisture stress tolerance of the bentgrass once the LDS formed.**

The preferred construction of golf putting greens is a 30-cm layer of properly graded sand (root zone) over a subdrainage system [U.S. Golf Association (USGA), 1993]. This ensures rapid water infiltration and drainage, provides resistance to compaction and facilitates maintenance of a smooth playing surface. The sand may or may not be amended with up to 20% by volume of organic material to improve moisture and nutrient retention (McCoy, 1992). A common organic amendment is sphagnum peat moss, which is considered a low quality carbon resource for soil microbes. Whether amended or not, soil aggregation does not occur in sand-based putting greens as in a native soil system, decreasing microenvironments for microbes. Ranjard et al. (2000) demonstrated that sand macroaggregates only contained 8.1% of the total bacteria associated with all sizes of aggregates, suggesting that the nutrient status of sand fractions (2.0 to 0.005 mm) is a limiting factor for bacterial colonization (Nunan et al., 2003). Consequently, newly constructed putting greens have been considered inhospitable environments for microorganisms as compared to native soils (Hodges, 1990; Mancino et al., 1993). Subsequently, broad generalizations have been made suggesting that putting green quality may suffer in the absence of the positive influences that an active microbial community can have on plant health (Hodges, 1990). More recently, Feng et al. (2002) showed bacterial counts for sand-based putting greens to be on the order of 10^9 cells/g of soil, which is in the magnitude of native bacterial community densities. However, the establishment and maintenance of root-zone microbial communities has been shown to relate to the age of the putting green (Aslan et al., 1999; Bigelow et al., 2002; Elliot et al., 2004). Bigelow et al. (2002), observed development of a large (10^8 cfu/g of dry soil) and relatively stable microbial community over the 2-year period following construction of a sand-based green. After construction, the putting green microbial communities undergo gradual shifts in composition and density (Elliot and Des Jardin, 2001; Elliot et al., 1999, 2004). Even though more data has been collected, there are many unanswered questions about how the microbial communities interact with established stands of creeping bentgrass.

The soil microbial community and higher plants have intricate relationships that are both mutually beneficial and competitive. Plant growth can be stimulated by rhizosphere microorganisms that are capable of producing plant growth regulators (Martens and Frankenberger, 1993; Timmusk et al., 1999). Pathogen damage to turfgrass can be reduced by disease suppressing microorganisms (Kageyama and Nelson, 2003; McKellar and Nelson, 2003; Nelson and Boehm, 2002; Nelson and Craft, 2000; Viji et al., 2003). However, in most instances the primary benefit of an active rhizosphere community to the plant community results from mineralization of organic substrates (Tate, 1995).

The turf industry has responded by marketing a wide array of biostimulants, which are loosely defined as turf amendments composed of natural wetting agents, seaweed extracts, plant hormones and microbial inoculums (Karnok, 2000). Some biostimulant products claim to increase soil microbial density and activity, which in turn enhances turfgrass quality due to increased organic matter decomposition and improved nutrient availability. These assumptions are based on previous research in other plant systems, which has shown that rhizosphere microbial activity can positively influence plant growth rates by increasing nutrient availability, inducing plant hormone-like compounds and reducing colonization of pathogens (Craft and Nelson, 1996; Groger, 1992; Hoflich et al., 1994; Paul and Clark, 1989). Chen et al. (2002) demonstrated increased dehydrogenase activity 1 week after the addition of two different agricultural amendments to soil microcosms, which indicates that the microbial community was stimulated by the addition of easily available carbon sources and other nutrients. Comparable studies have not been carried out in sand-based root-zone mixtures or in the putting green environments. The purpose of the present study was to determine how a select group of commercial biostimulants may alter the microbial community in a sand-matrix putting green and to link these changes to the quality of the creeping bentgrass playing surface.

Materials and Methods

Field experiment. The research site was a sand-based putting green constructed in 1996 at the University of Wisconsin O.J. Noer Turfgrass Research Station in Verona. The putting green was constructed according to USGA (1993) standards with a commercial

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root-zone mixture consisting of 80% sand: 20% sphagnum peat humus. Plastic sheeting (6 mm) was installed to the full depth of the root-zone mix to isolate the 1.8 × 2.4 m plots in the putting green. 'SR1119' creeping bentgrass [*Agrostis stolonifera* var. *palustris* (Huds.) Farw.] was grown-in during Spring 1997. The present study was initiated in Spring 2000, as the putting green was entering its third year after grow-in.

The five commercial biostimulants tested in this study were Flexx-Plus, Colonize T&O (Plant Health Care, Inc., Pittsburgh, Pa.), Experimental Microbial Stimulant A (Expt. A), Experimental Microbial Stimulant B (Expt. B), and Raiz-Mor. Flexx-Plus (Plant Health Care, Inc.) contained yucca wetting agent, beneficial bacteria, sea kelp, humic and fulvic acids. Colonize T&O was a combination of vesicular arbuscular mycorrhizae (VAM) fungi, bacteria (10^9 cfu/2.2 kg), humic acids, sea kelp, and carbon sources for bacteria. Exp A and B (Ocean Organics, Waldoboro, Mass.) contained seaweed extracts fortified with glucose. Raiz-Mor (Jay-Mar, Inc., Plover, Wis.) is sold as a surfactant–wetting agent containing seaweed and plant extracts. All treatments were analyzed for their mineral nutrient contents (Table 1) because biostimulants labeling is often incomplete for nutrient contents.

Four of the products, Flexx-Plus, Colonize T&O, Expt. A and Expt. B were applied as solutions with a CO_2 backpack sprayer calibrated to deliver 571 L·ha^{-1}. Per the manufacturer's recommendation, the Raiz-Mor was coated on granular fertilizer at the rate of 946 mL per 113.4 kg of fertilizer. Rates of application of the other products were Flexx-Plus at 38.3 kg·ha^{-1}, Colonize T&O at 2.78 kg·ha^{-1}, and Experimental A and B at 92.7 mL·ha^{-1}. The biostimulants were applied biweekly from 29 May until 21 Aug. 2000. A complete fertilizer, IsoTek 18N–1.31P–13.3K (Lebanon Turf, Lebanon, Pa.), served as the control and was applied biweekly at the rate of 12 kg·ha^{-1} nitrogen to all plots following the same schedule as the biostimulants. The treatments were randomized in a complete block experimental design with four replications.

Routine maintenance was similar to that of golf courses in the area of Madison, Wisconsin. The bentgrass was mowed 6 times a week at a height of 4.0 mm and clippings removed. Pesticide applications (chlorothalonil and chlorpyrifos) were on a curative basis for dollar spot (*Sclerotina homoecarpa*) and black cutworm (*Argoispiolon hufnagel*). The putting green was groomed and lightly sand top-dressed on a monthly basis. The creeping bentgrass was irrigated daily at 100% of the estimated plant ET (Diak et al., 1998). The putting green was aerified once with 64 mm (¼-inch) diameter quadratines to a depth of 5.0 cm on 5 × 5 cm spacings.

Soil samples were removed about every 2 weeks (22 May, 12 June, 28 June, 10 July, 24 July, 7 Aug., 21 Aug., 5 Sept., 18 Sept., and 2 Oct.) for a total of ten times by taking twelve randomly chosen cores of 1.25 cm in diameter to a 15.24-cm depth. The soil cores were processed by first removing the mat layer (topdressing sand intermingled with thatch and stolons) and then homogenizing the soil sample (Elliot et al., 1998). The soil samples were stored at 4 °C until further processing and analysis.

Turfgrass quality was visually assessed biweekly by rating for color, grass uniformity, and abundance of localized dry spot (LDS). The standard rating scale of 1 to 9 (Skogley and Sawyer, 1992) was used. A rating of 6.0 was set as representing a minimally acceptable value from the perspective of putting green performance. A single person performed all quality measurements to ensure the highest level of consistency between the individual ratings. The three ratings, all considered to be elements of putting green quality, were combined into a single composite value for statistical purposes. Bentgrass clippings were collected three times during the growing season and analyzed for total essential nutrient content to check possible influences of the biostimulants on the nutritional status. Clippings were oven-dried at 70 °C for about 24 h and then analyzed for percent nitrogen content following the method of Bremner (1965). All other nutrients were measured by inductively coupled plasma optical emission spectrometry on samples digested according to the method of Huang and Schulte (1985) at University of Wisconsin–Madison Soil and Plant Analysis Laboratory.

Microbial activity and biochemical assessment. Microbial activity was assessed by measuring four extracellular soil enzymes: dehydrogenase, cellulase, invertase and xylanase. Dehydrogenase, an overall indicator of microbial activity (Dick, 1997) was measured by the colorimetric procedure wherein triphenyl tetrazolium chloride (TTC) is reduced to triphenyl formazan (TPF) (Casida et al., 1964). Cellulase, invertase and xylanase activities were measured to assess microbial carbon cycling and biomass turnover within the root-zone using the methods developed by Schinner and von Mersi (1990). Soil enzyme activity was measured for all sampling dates.

Substrate utilization patterns were determined using Biolog Gram-negative plates (Biolog, Haywood, Calif.). The carbon substrate plates have been used to distinguish differences in microbial communities associated with different plant communities and arising from additions of various organic soil amendments (Burkett and Dick, 1998; Grayston et al., 1998). Substrate utilization profiles were assessed five times using soil samples collected on 22 May, 28 June, 24 July, 21 Aug., and 2 Oct. 2000. The soil samples collected 22 May before any biostimulant application provided background data for all comparisons. Briefly, 10 g (dry weight) of soil were added to 90 mL of sterile saline buffer and shaken for 1 h at 200 rpm. A 100-µL sample from the 10^{-3} soil extract dilution was added to each well in the Biolog gram-negative microplates (Balser, 2000; Biolog, 1993; Zak et al., 1994). The microplates were incubated at 30 °C in an airtight container with extra water to reduce plate evaporation. Color development of the Biolog plates was read four times at 12-h intervals after the initial 4-h background reading. Plate readings from the 48-h time point were used for all analyses after noting that after this time there were no further positive reactions for any of the substrate. Readings were collected with a MRX Microplate Reader (Dynateck Laboratories, Inc., Borehamwood, U.K.) set at 590 nm to measure color development. Bio Linx: Assay Management software version 2.22 (Dynex Technologies, Inc., Chantilly, Va.) was used to evaluate the data.

Statistical analysis. Data analyses were performed using SAS Version 8 (SAS Institute Inc., Cary, N.C.). The data collected in the biweekly sampling schedules were consolidated into 10 cycles. All data except the substrate utilization profiles were analyzed with a repeated measurements procedure. Student's *t* tests and least squared means (LSM) were used to compare the treatments, date, and the treatments by date interactions for significant differences. Substrate richness was calculated by determining the percentage of the 95 carbon substrates oxidized (Ellis et al., 1995). Applying the criteria of Burkett and Dick (1998), each well with absorbency over 0.5 was counted as a positive response. The number of positive responses at 48 h was divided by the number of carbon sources on the plates to determine the percent of carbon substrates used. The raw data from the Biolog-Gram negative plates were used to calculate the average well color development (AWCD) for each plate (Garland and Mills, 1991). The 95 carbon substrates were then separated into 6 guilds: carbohydrates, carboxylic acids, polymers, amino acids, amines/amides, and miscellaneous based on a substrate evenness analysis developed by Zak et al. (1994). Using the carbon substrate guilds reduces the amount of information lost when a single value is derived from all 95 carbon substrates. The mean value of each guild was used in a MANOVA analysis. Substrate richness and carbon substrate guild data were evaluated by least square means with student *t* test used to determine significance. Interactions between turfgrass visual quality and the microbial community indicators were evaluated by way of Pearson correlation coefficients.

Table 1. Mineral analysis of the commercial biostimulant products.

	Element in biostimulant (%)			
Products	Nitrogen	Phosphorus	Potassium	Iron
Flexx-Plus	0.08	0.20	8.84	4.81
Colonize T&O	0.07	2.58	9.32	0.02
Experimental A	2.92	0.03	1.08	2.35
Experimental B	3.19	0.02	0.94	2.06
Raiz-Mor	1.51	0.13	0.02	0.001

Results

Microbial activity and biochemical assessment. The activities of the four edaphic enzymes measured in this study were uniformly very low as compared to cropland soils. For example, the dehydrogenase and invertase levels measured were 100 to 200 times lower than those reported by Pickel and Hayes (1990) for soil collected from Indiana farms with corn-soybean rotations. One very plausible reason for this sharp contrast in levels of microbial activity is that the sand-based root zone material that was in this study was strongly substrate limiting. According to Tate (1995), restrictions in carbon resource are what most commonly controls soil microbial community development. Therefore, the application of a biostimulant with energy rich carbon would appear to have considerable potential for enhancing microbial activity for short periods of time.

The biostimulant treatments either never altered enzyme activity or did so only temporarily. When compared to the control (fertilizer only) treatment, significant changes in dehydrogenase activities ($p < 0.01$) were sporadic, occurring in the Flex-Plus treatment on 28 June and 24 July, 28 June for Colonize T&O and Expt. A, and 10 July for Raiz-Mor (Fig. 1). Cellulase (Fig. 2), invertase, and xylanase activities (data not shown) were never significantly increased as a result of biostimulant application.

The most pronounced change in enzyme levels that was highly significant ($p = 0.01$) was the downward trend in cellulase activity as the growing season progressed (Fig. 2). This is indicative of a progressive decline in substrates originating from sources other than the organic compounds being applied.

Bacterial substrate utilization patterns were also used to detect changes in indigenous bacteria activity. A larger percent of positive wells for the treatments compared to the control was used to indicate increased metabolic capabilities of the bacterial community. The percent positive wells ranged between 45% to 54% for the control treatment and 37% to 54% for the biostimulants (Table 2). With just two exceptions for 25 observations, there were no repeated significant differences across dates between the percentage of positive wells in the biostimulant treated soils and the untreated control.

In an effort to more clearly identify any divergences in the microbial communities as a result of biostimulant application, the 95 Biolog carbon sources were divided into six different carbon guilds. Statistical analyses indicated that there were few significant changes in carbon guild utilization from the beginning to the end of the season (Table 3). The few significant treatment effects noted occurred sporadically across the five sampling dates and provided no hard evidence that the biostimulants induced sustained changes in the composition or metabolic activity of the microbial communities.

Turfgrass quality. While the biostimulants had no noteworthy influences on soil microbial activity or composition of the microbial community, in most cases they did improve the visual quality of the turfgrass (Table 4). The one exception was a decrease in turfgrass quality observed after an excessive rainfall event (6.2 cm) on 13 June 2000. Increased turfgrass quality was sustained for 30 d after biostimulant and fertilizer application ended on 21 Aug. Ratings began to decline shortly after 12 Sept. Significant improvements in turfgrass quality were repeatedly measured for all biostimulant treatments across all sampling cycles. An interaction between the treatment effects and sampling cycles proved to be significant ($p < 0.05$), indicating that the treatment effects out-performed natural variation in the system.

All individual measurements of turfgrass quality (e.g., color, stand uniformity, and the occurrence of localized dry spot) were positively influenced by the biostimulants (Table 5). However, it was the percentage of plot area with

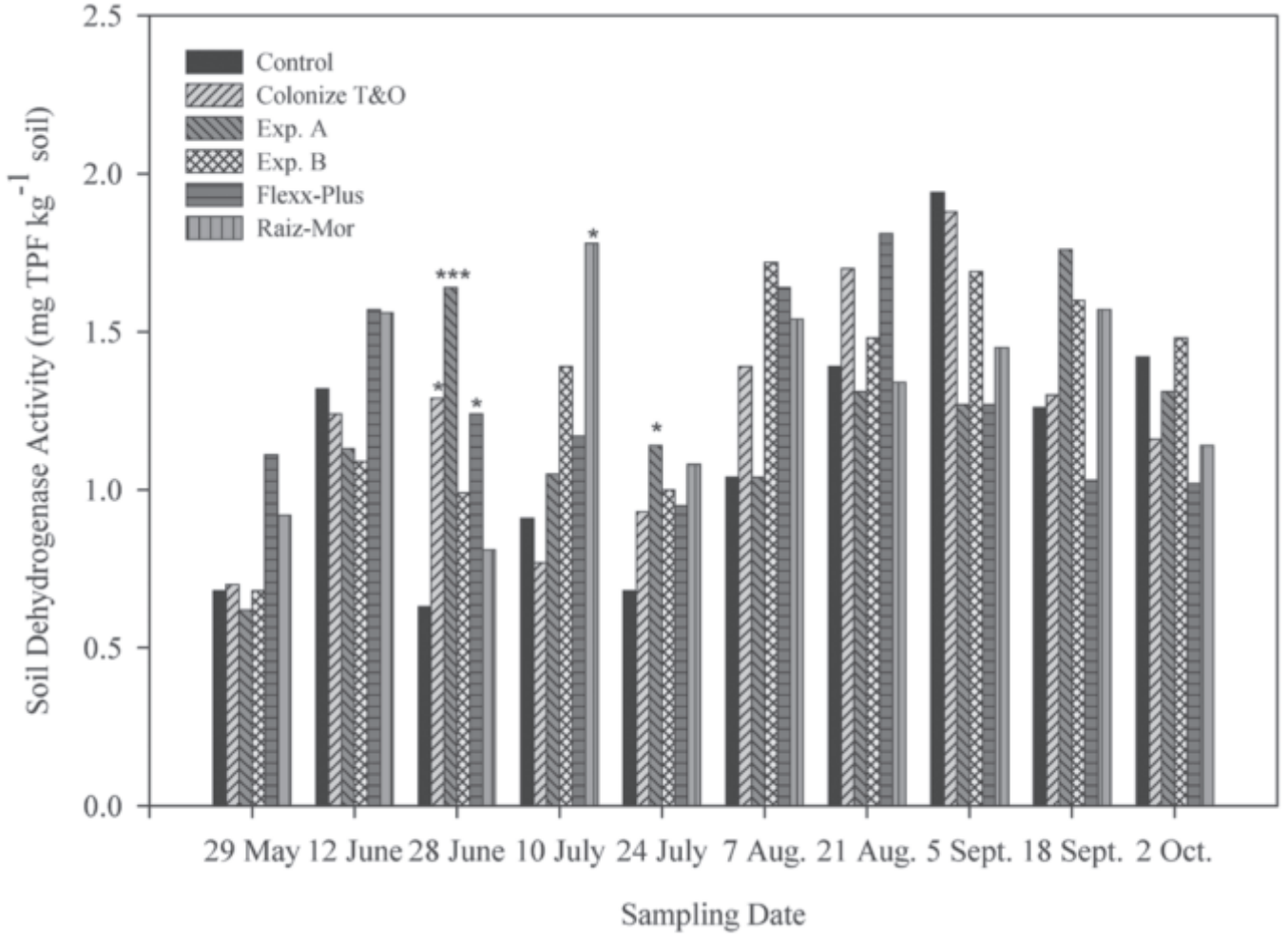

Fig. 1. Seasonal changes in soil dehydrogenase levels following the application of five different commercial biostimulants.*,**,***Significant at $p = 0.10$, 0.05, and 0.01, respectively for each treatment as compared to the control. Each column is a mean of three replicate measurements.

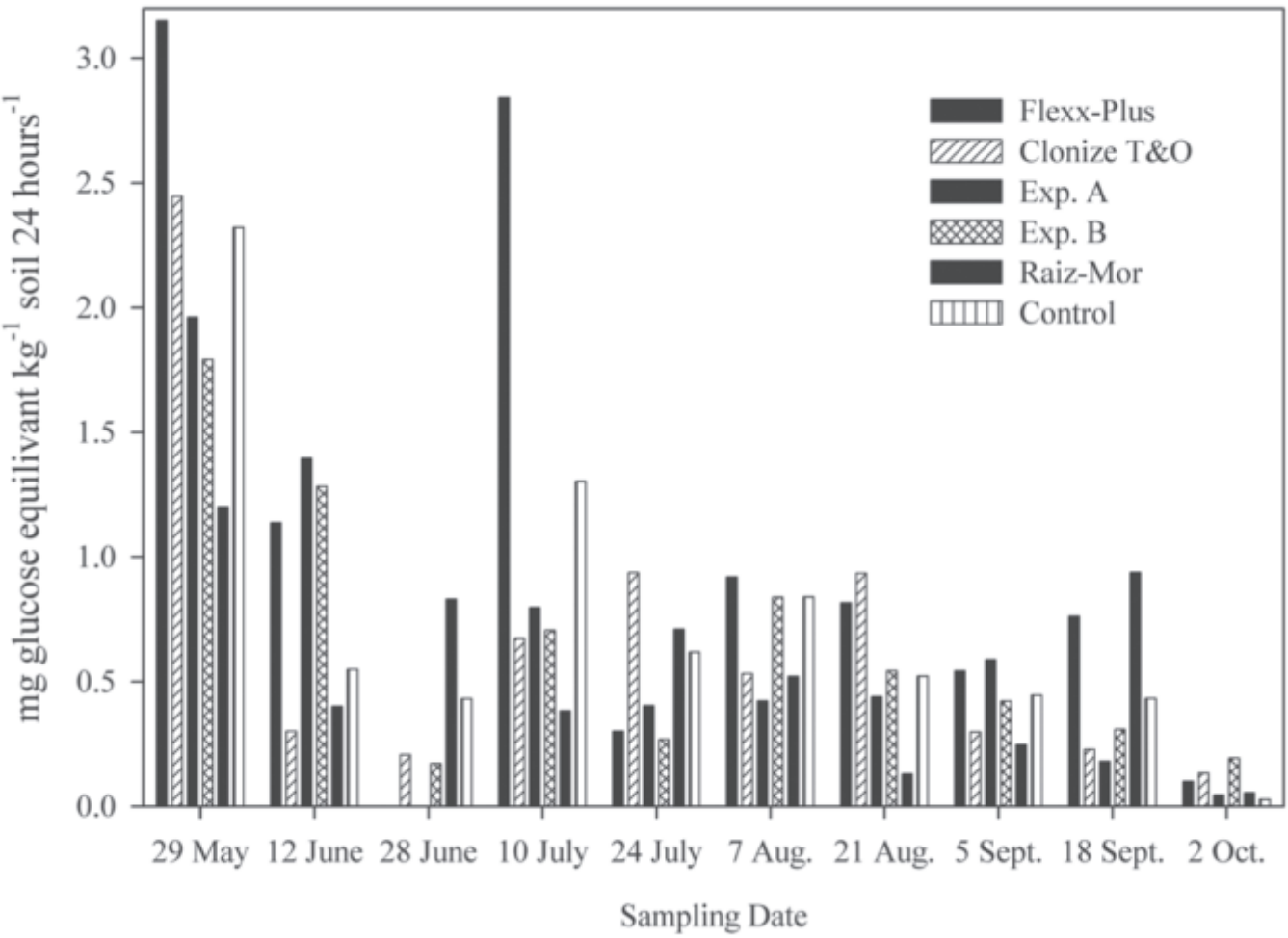

Fig. 2. Cellulase activity determined colorimetrically for the root-zone of a U.S. Golf Association style sand-based putting green over the 2000 field season following biweekly applications of commercial biostimulants and IsoTek 18N–1.31P–13.3K complete fertilizer.

Table 2. Substrate richness for bacterial communities determined by carbon utilization patterns and the percentage of positive reactions from Biolog Gram-negative plates for five soil sampling dates over the 2000 field season.

	Positive wells (%)				
	Sampling date				
Treatment	29 May	28 June	24 July	21 Aug.	2 Oct.
Flexx-Plus	51.2	47.0	51.0	44.3	50.8
Colonize T&O	48.2	52.3**	50.5	46.1	53.2
Experimental A	50.3	41.1	54.7	47.7	52.2
Experimental B	48.5	52.9***	49.2	48.3	51.9
Raiz-Mor	48.9	37.1	53.7	46.0	51.4
Control	49.5	45.0	54.1	47.1	53.4

*,**,***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Contrasts of biostimulant treatments against the control.

LDS that was most significantly decreased by the treatments. The treatments significantly reduced LDS ($p < 0.05$) for each observation date and the differences remained significant ($p < 0.10$) when seasonal trends were taken into account. These reductions in the extent of hydrophobic areas on the greens appear to provide the best explanation for biostimulant induced improvements in putting green quality.

Treatment effects on turfgrass plant nutrition did not explain improvements in turfgrass quality. Nutrient levels in the creeping bentgrass did not vary significantly over the season (Table 6). The levels of tissue nitrogen, zinc, manganese, and iron concentrations for 19 June were significantly increased compared with the control. However, this trend was not sustained for more of the nutrients (except Mn for Expt. B) in a subsequent sampling date. In the 18 Sept. tissue samples, Ca and Mg concentrations were higher than the control for all treatments. However, the other nutrient levels fell within sufficiency ranges for healthy turfgrass. Nitrogen availability may have played a role in intensifying color during the early part of the field experiment. Even with the biostimulants containing nitrogen at concentrations as high as 3% and potassium up to 9% in their formulations, the total quantities of nutrients applied via the biostimulants never exceeded 0.025 kg·ha^{-1}.

The visual quality of the turfgrass was related to the microbial community measurements by way of a correlation matrix. There were no significant relationships between the application of biostimulants and the microbial community (data not shown).

Discussion

The commercial biostimulants tested in this field experiment significantly improved the visual quality of creeping bentgrass, but the treatments did not effectively alter the putting green microbial community in terms of enzyme activities and substrate utilization. In contrast, a study by Chen et al. (2002) revealed that commercial biostimulants possess the ability to increase microbial activity. In that study, the soil had been air-dried, sieved and stored for a year before use in microcosm experiments. Application of biostimulants would have provided an easily degradable source of organic matter for the dormant microorganisms and mostly likely explains the differences in dehydrogenase levels measured. In the present study, a mat layer (thatch and stolons intermingled with topdressing sand) was in place when the biostimulants were applied. The biostimulant carbon sources may not have migrated below this about 1.5-cm-thick layer, which was removed before processing the soil cores. Mat layer removal limited the detection

Table 3. Biostimulant effects on soil microbial community divergence as indicated by Biolog Gram-negative plate carbon guild substrate evenness.[z]

		Substrate evenness					
		Treatment					
Date	Carbon guild	Flexx-Plus	Colonize T&O	Experimental A	Experimental B	Raiz-Mor	Control
22 May	Carbohydrate	0.700	0.585	0.599	0.666	0.673	0.665
28 June	Carbohydrate	0.567	0.721*	0.751**	0.366**	0.342**	0.559
24 July	Carbohydrate	0.670***	0.718**	0.627***	0.830	0.806	0.826
21 Aug.	Carbohydrate	0.531	0.521	0.527	0.541	0.561	0.578
2 Oct.	Carbohydrate	0.643***	0.706**	0.720*	0.689**	0.732	0.792
22 May	Carboxylic acids	1.075	1.014	1.058	1.100*	1.070	0.981
28 June	Carboxylic acids	1.003	1.071**	1.081**	0.802**	0.705***	0.942
24 July	Carboxylic acids	0.947***	0.933***	1.012***	1.147	1.103	1.148
21 Aug.	Carboxylic acids	1.010	0.980	1.011	1.078	1.024	1.040
2 Oct.	Carboxylic acids	1.018	1.062	1.042	1.055	1.056	1.069
22 May	Polymers	0.489	0.489	0.436*	0.493	0.576	0.516
28 June	Polymers	0.662	0.725**	0.757***	0.564	0.459	0.572
24 July	Polymers	0.593	0.538	0.521	0.775***	0.588	0.606
21 Aug.	Polymers	0.545	0.533	0.542	0.558	0.554	0.519
2 Oct.	Polymers	0.566	0.568	0.607	0.640	0.626	0.564
22 May	Amines	0.604	0.602	0.534	0.601	0.422	0.558
28 June	Amines	0.638**	0.690***	0.814***	0.498	0.409	0.456
24 July	Amines	0.454*	0.408***	0.412***	0.681	0.568	0.598
21 Aug.	Amines	0.391	0.402	0.483	0.430	0.404	0.414
2 Oct.	Amines	0.622	0.590	0.642	0.633	0.708**	0.472
22 May	Amino acids	1.154	0.980	1.035	1.156	1.003	1.140
28 June	Amino acids	1.255	1.357***	1.470***	1.173	0.841***	1.113
24 July	Amino acids	1.023***	0.986***	0.941***	1.323**	1.123	1.193
21 Aug.	Amino acids	0.820	0.959	0.910	1.016	0.978	0.912
2 Oct.	Amino acids	1.147	1.197	1.231	1.211	1.200	1.213
22 May	Miscellaneous	0.503***	0.520***	0.476**	0.465**	0.454**	0.353
28 June	Miscellaneous	0.423**	0.439***	0.555***	0.334	0.272	0.320
24 July	Miscellaneous	0.539	0.546	0.440	0.624**	0.603	0.508
21 Aug.	Miscellaneous	0.380	0.477	0.452	0.624	0.406	0.435
2 Oct.	Miscellaneous	0.497	0.493	0.438**	0.457	0.445	0.500

[z]Substrate evenness: E = H'/Hmax, H' = –Σ pi (ln pi), Shannon index of diversity value; pi = proportion of color development for i[th] well.
*,**,***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively as compared to control.

Table 4. Creeping bentgrass (*Agrostis stolonifera* L.) 'SR 1119' visual quality ratings (e.g., the composite of color, percentage of localized dry spot, and turfgrass uniformity) following repeated applications of five biostimulant treatments starting at 29 May and continuing until 21 Aug.

	Quality rating									
	Observation day									
Treatment	29 May	5 June	19 June	10 July	24 July	1 Aug	16 Aug.	29 Aug.	12 Sept.	2 Oct.
Flexx-Plus	7.2	7.3***	6.4***	7.1**	7.6***	8.0**	7.4	7.9	7.9	7.2
Colonize T&O	6.5	6.4	5.1	7.2**	7.5**	7.8*	7.6	7.8	7.8	6.8
Experimental A	6.8	7.1**	6.5***	7.4***	7.5***	7.9**	7.7	7.9	7.9	7.0
Experimental B	7.2	7.1**	6.7***	7.5***	7.5***	8.0**	7.3	8.0	7.9	7.3
Raiz-Mor	7.3	6.9	6.4***	7.6***	7.7***	8.0**	7.8**	8.0	8.0	7.2
Control	6.2	6.4	5.1	6.6	6.7	7.3	7.5	7.7	7.6	6.8

*,**,***Contrasts of biostimulant treatments against the control significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 5. Visual localized dry spot ratings for creeping bentgrass (*Agrostis stlonifera* L.) 'SR 1119' after repeated biweekly applications of the five biostimulant treatments applied at manufacturers rates starting at 29 May and continuing until 21 Aug.

	LDS rating[a]									
	Observation day									
Treatment	29 May	5 June	19 June	10 July	24 July	1 Aug	16 Aug.	29 Aug.	12 Sept.	2 Oct.
Flexx-Plus	7.6***	8.0	7.4**	7.4	7.9*	7.8	7.5	8.5	8.5	7.9
Colonize T&O	5.9	7.3	5.5	7.9*	8.1**	8.1	8.2	8.2	8.4	7.7
Experimental A	6.5**	8.3	7.5**	8.0*	8.0*	8.1	8.1	8.4	8.5	7.9
Experimental B	7.5***	8.4*	7.9***	8.0*	8.5***	8.4	7.5	8.5	8.4	7.9
Raiz-Mor	7.8***	8.6**	7.4**	8.4***	8.6***	8.3	8.3	8.5	8.8	8.0
Control	5.1	7.4	6.1	7.0	6.9	7.5	7.9	8.0	8.0	7.3

[z]LDS Rating based on a scale of 1 to 9, with 9 = a green without any areas of LDS.
*,**,***Contrasts of biostimulant treatments against the control significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

of more pronounced biostimulant influences on microbial activity. In a turfgrass setting, Feng et al. (2002) demonstrated that the plant growth regulator, trinexapac-ethyl, did not alter the turfgrass microbial community. These results suggest permanent plant cover limits the benefits of foliar applied products upon soil microbial communities. Additionally, Sigler et al. (2001) successively added *Pseudomonas aureofaciens* TX-1 to creeping bentgrass; this did not change community composition as detected by a molecular analysis. Foliar applications of large bacterial densities do not change the indigenous soil microbial community, and suggesting that products containing beneficial bacteria in the formulation would not have altered community structure.

Substrate utilization patterns gave no indication that significant shifts in the putting green microbial communities were triggered by the biostimulants. Instead, the results indicate possible spatial differences within the putting green environment because of how the percentage of carbon substrate utilization varied slightly but insignificantly between treatments. Only fast growing bacteria are known to respond quickly to the high nutrient levels in Biolog

Table 6. Plant essential nutrients in creeping bentgrass (*Agrostis stolonifera* L.) leaf tissue from the 2000 season.

Date	Treatment	N	P	K	Ca	Mg	S	Zn	B	Mn	Fe	Cu
		(mg·kg^{-1})			(g·kg^{-1})					(mg·kg^{-1})		
19 June	Flexx-Plus	4.35	3.08	---	4.13	2.89	3.73	40.7	6.55	42.3	237	20.1
19 June	Colonize T&O	4.31	3.08	---	4.11	2.86	3.59	39.2	6.12	43.9	297	22.0
19 June	Experimental A	4.44	3.11	---	4.02	2.78	3.72	43.6	6.75	44.9	287	22.9
19 June	Experimental B	4.45	3.03	---	4.07	2.83	3.76	43.5	7.62	49.5	280	20.0
19 June	Raiz-Mor	4.38	3.15	---	4.13	3.00	3.89	42.2	7.15	41.4	241	20.2
19 June	Control	4.14	3.11	---	3.97	2.85	3.56	46.8	6.40	44.7	532	32.5
LSD		0.05	NS	---	NS	NS	NS	0.05	NS	0.05	0.05	NS
14 Aug.	Experimental A	4.14	4.82	18.8	3.91	3.20	4.22	43.0	6.02	26.5	256	22.5
14 Aug.	Experimental B	4.11	4.57	18.1	3.81	3.09	4.07	41.3	5.36	27.7	473	20.2
14 Aug.	Colonize T&O	4.14	4.61	18.4	3.82	3.11	4.13	39.5	5.62	30.1	419	20.6
14 Aug.	Flexx-Plus	4.09	4.51	18.1	3.83	3.10	4.09	39.9	4.98	26.2	352	19.8
14 Aug.	Raiz-Mor	4.31	4.83	---	3.87	3.14	4.35	41.4	5.09	24.8	230	21.2
14 Aug.	Control	4.27	4.44	17.6	3.57	2.93	3.98	38.7	5.14	29.6	551	21.1
LSD		NS	NS	NS	NS	NS	NS	NS	NS	0.05	NS	NS
18 Sept.	Experimental A	3.70	4.21	17.6	4.29	3.01	3.41	37.1	4.65	25.1	285	17.6
18 Sept.	Experimental B	3.60	4.03	17.1	4.40	3.08	3.35	36.8	4.82	26.4	327	17.0
18 Sept.	Colonize T&O	3.55	4.14	17.6	4.22	3.02	3.41	35.8	5.49	28.4	361	17.6
18 Sept.	Flexx-Plus	3.43	3.92	16.8	4.23	2.95	3.24	36.5	4.00	25.7	345	16.7
18 Sept.	Raiz-Mor	3.71	4.33	17.7	4.40	3.05	3.48	38.2	4.25	23.9	248	17.5
18 Sept.	Control	3.51	4.19	17.6	4.06	2.93	3.46	36.5	5.79	28.1	366	18.0
LSD		NS	NS	NS	0.05	0.05	NS	NS	0.05	NS	NS	NS

[NS]Nonsignificant.

wells (Degens et al., 2000; Garland and Mills, 1991), which suggests only a small percentage of the populations were measured using this technique. However, if one assumes that the primary carbon resources in this study were root exudates, this may not be true by virtue of the fact that root exudates stimulate primarily fast growing microbes (Tate, 1995). The differences observed between the treatments and control did not suggest an altered population of fast growing bacteria in the root-zone. The results also indicated that the carbon compounds contained in the commercial products were easily degradable and could be utilized by the indigenous community without acquiring new metabolic capabilities.

Response of the creeping bentgrass to the biostimulants was judged not to be nutritional in origin because the nutrient concentrations in the bentgrass leaf tissue were not altered significantly. Furthermore, all nutrient levels fell within the sufficiency ranges for healthy creeping bentgrass (McCarty, 2001). The most plausible explanation for increased turfgrass quality in our study are that different components in the biostimulants reduced localized dry spot and, in turn, may have increased the heat and moisture stress tolerance of the creeping bentgrass stand. Decreases in the incidence of localized dry spot have been achieved with commercial surfactants, the result being higher creeping bentgrass quality during summer heat stress (Kostka, 2000). Zhang et al. (2003) showed that both humic acids and seaweed extracts can improve turfgrass quality during July, an early part of summer decline. In this study, two of the biostimulant products contained wetting agents and the other three products contained some type of seaweed extract or humic acid, which most likely explains any increases in turfgrass quality.

Perhaps the most important observation from this field study was the decreases in soil enzyme activity as the season progressed. This trend suggests that in the 3-year-old putting green studied, microbial community activity was governed by something other than the physical soil environment or the products applied to the system. Creeping bentgrass responds negatively to high ambient air and soil temperatures (Huang and Gao, 2000; Huang and Xu, 2003; Liu and Huang, 2000). When creeping bentgrass is under heat stress, net photosynthate production declines and fixed carbon is preferentially partitioned to stems and shoots rather than roots (Xu and Huang, 2001). Huang and Xu (2003) demonstrated that the total amount of carbon in the root systems decreases during the hotter months (May to August) and then increases when temperatures decline. Soil temperatures in our putting green over July to August ranged from 22 to 26 °C, which are above the optimum temperature for creeping bentgrass. Bokhari and Singh (1974) have demonstrated that temperature suppression of grass growth is accompanied by reductions in total root exudate production, not because exudate production per unit of root changes, but because of root mortality. This supports the suggestion of Bigelow et al. (2002), that reductions in substrate availability decrease total microbial numbers in sand-based turfgrass stands. Additionally, Feng et al. (2002) presented evidence that root die back was responsible for lower total bacteria in cool season turfgrass than in a warm season turf stand. Indeed, Farrar et al. (2003) have expressed the view that amounts of exudates released by plant roots are a controlling factor in the activity of soil microbial communities.

Declining quantities of root exudates associated with heat stress are clearly a plausible explanation for the decreases in microbial activity observed over time in this study. This implies is that the carbon substrates applied via the biostimulants were inconsequential with regard to alteration of root zone microbial activity. Rather, our observations suggest that it was reductions in LDS that increased turfgrass visual quality.

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