ENVIRONMENTAL AND MICROBIOLOGICAL CHARACTERISTICS OF MANAGED URBAN TURFGRASS SYSTEMS

A Thesis

by

STEPHEN ZACHARY CASTER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Soil Science

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Approved by:

Co-Chairs of Committee,	Terry Gentry
	David A. Zuberer
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ABSTRACT

Environmental and Microbiological Characteristics of Managed Urban Turfgrass Systems. (May 2009)

Stephen Zachary Caster, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. David A. Zuberer
Dr. Terry Gentry

A two-year study was conducted in which soil solution, drainage water, and soil core samples were taken from three turfgrass test plots and two sports fields, located on the Texas A&M University campus. The goal was to determine concentrations of organic nutrients moving through the soil profile, as well as to characterize microbial numbers and community composition under three turfgrasses on a native soil (Boonville fine sandy loam) and two sports fields with highly engineered sand-based root zones. The turfgrasses were Bermudagrass (*Cynodon dactylon* (L.) Pers), Zoysiagrass (*Zoysia japonica* Steud.), and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze). The sports fields (soccer/softball) were sodded with 419 turf-type Bermudagrass and over-seeded in winter with ryegrass (*Lolium perenne* (L.)). Soil solutions (turfgrass plots) or drainage water (sports fields) were analyzed for dissolved organic carbon (DOC) with a total organic carbon (TOC) analyzer; for dissolved organic nitrogen (DON) using a persulfate oxidation method; and for dissolved organic phosphorus (DOP) with an ascorbic acid method.

Microbial populations were characterized with respect to bacterial and fungal numbers by standard plate counts; carbon substrate utilization (CSU) with Biolog® GN2 plates; and community composition with a fatty acid methyl ester (FAME) protocol.

Nutrients in soil solutions varied between individual plots, showing seasonal trends in DOC and DON, with peak concentrations of DOC (135.3mg L^{-1}) and DON (43.5mg L^{-1}) found during late summer. DOP levels were highly variable among the turf plots with a peak during early fall (11.6mg L^{-1}). Drainage water samples demonstrated similar seasonal trends in DOC and DON, with peak levels of DOC (118.4mg L^{-1}) and DON (58.5mg L^{-1}) also occurring during late summer. Concentrations of DOP were markedly lower in the drainage water than in the soil solution, with a peak in early fall (8.3mg L^{-1}).

Bacteria and fungi showed some minor seasonal changes throughout the year, but were not significantly different. CSU profiles demonstrated a high degree of overlap across all sites, indicating comparable metabolic capacity regardless of the type of turf. The FAME results identified differences in the microbial communities under the turf plots and sports fields, as evidenced by the high degree of separation observed between the FAME profiles from the Boonville soil and sand-based root zones. When FAME profiles were compared over time, there also appeared to be little overlap between the individual sampling dates at all locations, which may be an indicator of seasonal influence.

ACKNOWLEDGEMENTS

I would like to thank Dr. Zuberer and Dr. Gentry for both their patience and guidance throughout the study. I would also like to thank Dr. Hons and Dr. Provin for their insight and help with the initial start up of our study, as well as Annette Fincher, Heidi Mjelde, and Jeff Waskom for their help with the analysis of so many water samples. I would like to thank Dr. Boutton and Dr. McInnes for putting up with me in class and providing help outside the classroom, as well as all the other teachers I have had throughout my educational career.

I would also like to thank the Texas Turfgrass Association for financial support of my project through the Texas Turfgrass Research, Extension, and Education Endowment and the Texas Water Resources Institute (TWRI) for partial financial support of my project through a Mills Scholarship, as well as Leo Goertz and Craig Potts for allowing us access to the sports fields.

I would finally like to thank my mother and sister, who were always there to support me and remind me of where I came from, and what I can do.

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INTRODUCTION

Population growth and expansion of suburban development throughout the United States have led to less land availability for agriculture. The encroachment of metropolitan areas into agricultural lands has led to changes in nutrient cycles, which has in turn led to changes in ecosystem composition (McDonnell and Pickett, 1990). Soils that had for generations been subjected to continuous crop production are now being covered with both native and non-native turf species. Turfgrasses have the potential to prevent erosion, improve soil aggregation, provide small-wildlife habitats, improve the aesthetic qualities of the urban environment, and sequester carbon (C) (Beard and Green, 1994; Qian and Follett, 2002). While there has been extensive research into agricultural land management over long periods of time (i.e., studies of quantities of nitrogen (N) and phosphorus (P) lost from the soil profile due to runoff and leaching), less is known about the long-term influences of urbanization on organic nutrients, and the resultant influences on C, N, and P cycles in urbanized ecosystems. In urban landscapes, organic nutrients can be metabolized by the soil biota, tied up in soil microbial biomass, lost as runoff, leached through the soil profile, etc., just as in agricultural fields. However, agricultural landscapes typically consist of large fields, measured in acres, with generally uniform fertilizer and irrigation rates. Typical urban settings, such as home lawns and sports fields, are generally much smaller and may be subject to extreme variability in fertilization rates and irrigation practices within only a

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This thesis follows the style of the Soil Science Society of America Journal.

few feet of each other (i.e., neighbors in a residential district fertilize and irrigate at different rates). Golf courses, another component of the urban landscape, also present the difficulty of varying types of management practices (King et al., 2000), depending on the location relative to the course (i.e., putting greens and tees versus fairways and roughs). The large variability in management practices among these differing types of urban settings makes it difficult to relate prior agricultural soil research on quantities of organic forms of C, N, and P to the growing urban landscape. This leads me to question whether urbanization is truly beneficial or detrimental to the environment, specifically, with regards to organic nutrient enrichment of waterways through runoff and leaching. McDonnell and Pickett (1990) have summarized potential ecological and environmental changes that might arise with urbanization of a landscape (Fig. 1).

Also associated with organic nutrients, are the microbial populations that exist in both the soils and water of the urban environment. Recent research has demonstrated that soil microbial populations vary in community structure and functionality under disturbed and undisturbed areas of a golf course (Bigelow et al., 2002). In relative terms, this indicates that with different functionality, the potential for microbially mediated processes in the soil profile (e.g., methanogenesis, nitrification, and denitrification) changes depending on the location, which can have both positive and deleterious consequences. Organic C, N, and P also influence the microbial populations in waterways, by providing a food source for microbes that exist in an otherwise nutrient-poor environment. This has its own consequences with regards to pathogen survival and eutrophication (Bretter and Höfle, 1992; Carpenter et al., 1998b). Research

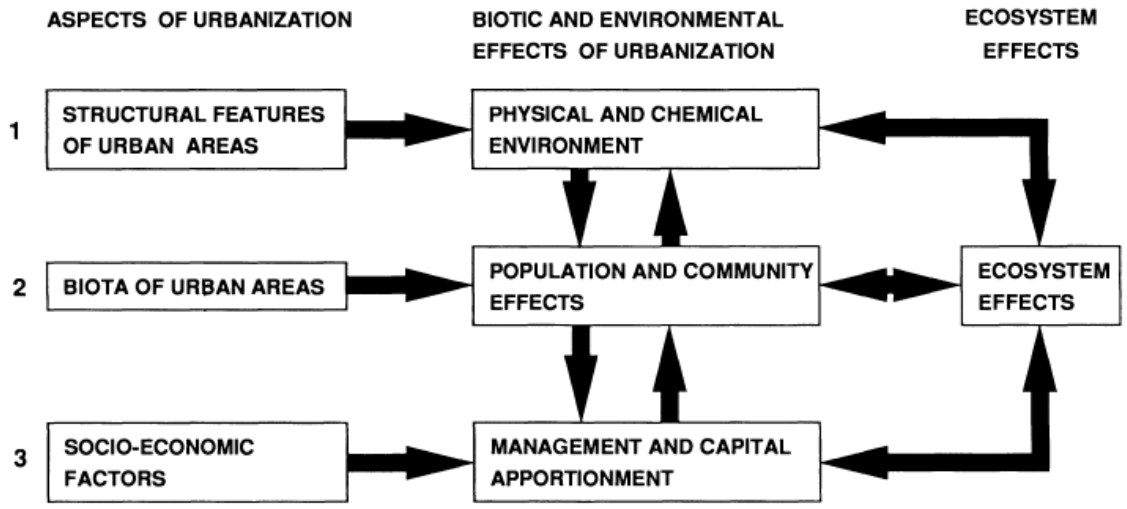

Fig. 1 - Effects of urbanization on the environment. Conversion of once rural areas into subdivisions and urban centers has led to the introduction of new structural features, biota, and economic factors. These aspects of urbanization influence the environment and can lead to changes in ecosystems (McDonnell and Pickett, 1990).

is needed to identify the changes that may be occurring in microbial populations due to alterations in quantities of organic C, N, and P, as the urban environment expands.

REVIEW OF LITERATURE

CURRENT EFFECTS OF URBANIZATION

The urban landscape is diverse, including residential lawns, city parks, sports fields, and golf courses. Each of these systems may include intensive management practices, such as fertilizer applications, frequent mowing at various heights, and highly variable irrigation rates. According to Qian and Follett (2002), an increasing percentage of land is being planted with turfgrasses throughout the United States, demonstrating the expanding nature of the metropolitan environment. Urban fertilizer application rates and frequencies are extremely variable depending on the individual land owners (Kaye et al., 2006). In the state of Texas alone in 2004, over $207 million dollars went towards fertilizer for urban land usage (Lard et al., 2005). Areas previously dedicated for agricultural uses have been turned into subdivisions, resulting in the reintroduction and restoration of some native species and ecosystems (Wuerthner, 1994). These residential districts can act as potential "sinks" for added nutrients, due to their highly centralized and efficient drainage systems (Walsh et al., 2005). This has its own ecological impacts with regard to nutrient cycling, such as the increased potential for higher denitrification rates (Zhu et al., 2004). Those same drainage systems can also aid in the movement of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP) from residential districts to various waterways. Each of these organic compounds has its own influences on nutrient cycling. Fluxes of additional organic nutrients to hydrological

environments can possibly accelerate the process of eutrophication in "natural" water ecosystems (Gjettermann et al., 2007; Carpenter et al., 1998a), as well as other disturbances to major nutrient cycles resulting in increased pathogen (i.e., *E. coli*) survival (Bretter and Höfle, 1992). While there has been extensive research into agricultural land management as a non-point source of pollution (i.e., fertilization effects on soil solution and potential runoff of DOC, DON, and DOP into water ecosystems) (Wuerthner, 1994), very few studies to date have focused on increased urbanization and possible offsite consequences. It is therefore important that we identify the potential for movement of DOC, DON, and DOP from these expanding metropolitan environments because of the influences of these organic nutrients on nutrient cycling and the subsequent effects on natural aquatic ecosystems and macro- and micro-fauna populations.

DISSOLVED ORGANIC CARBON

Dissolved organic carbon (DOC) represents a readily available form of carbon for microbial populations. Its relative ease for incorporation into cellular constituents makes DOC a prime candidate for consumption by prokaryotic populations (Carlson et al., 2004). As the urban landscape grows, runoff and leachate enter a network of drainage systems that flow towards a centralized drain output, which brings the combined drainage water together before entering into natural waterways. Increasing levels of DOC in these aquatic ecosystems provide microbial communities with an added carbon source and can simultaneously increase specific microbial processes such

as mineralization and denitrification (Vinther et al., 2006). Influencing these microbial processes will possibly alter both carbon and nitrogen cycles.

Global warming has become a central topic for scientists, land managers, and policy makers alike. There are growing concerns that the effects of carbon dioxide emissions are irrevocably altering the global carbon cycle (Alley et al., 2007). As such, the direction of future research funding and legislation is aimed at understanding processes and promoting policies that can reduce carbon dioxide emissions and potentially reincorporate carbon back into the terrestrial ecosystem (i.e., carbon sequestration). Increased levels of DOC in natural waterways can lead to higher rates of carbon sequestration. Introduced high-molecular weight (>10,000 mw) DOC can be easily broken down by bacterial populations into very recalcitrant low-molecular weight DOC (<10,000 mw) due to the adherence of labile organic carbon to the "outside" of larger polymers (Tranvik, 1990). These readily available substrates are utilized, and the remaining low-molecular weight DOC constituents can have an average turnover rate of thousands of years (Amon and Benner, 1996). A growing urban landscape might therefore potentially lead to higher carbon sequestration rates depending on the type of allochthonous DOC being introduced into natural waterways.

DISSOLVED ORGANIC NITROGEN

Dissolved organic nitrogen (DON) frequently represents a limiting factor for biological processes. As multiple land managers apply varying rates of nitrogen fertilizer, additional DON may be incorporated into soil solution. This will in turn

lower both the C:N ratio and immobilization rate of nutrients. With lower C:N ratios and increased nitrogen availability, both ammonification and nitrification (two important processes in the nitrogen cycle of soils) increase, and the added organic nitrogen compounds are broken down to form ammonia, then converted to nitrite and nitrate (Alef, 1995). These biologically available forms of nitrogen can be incorporated into cellular constituents or lost from the environment through several different mechanisms. In addition, increased levels of nitrate in aquatic ecosystems can lead to contamination of drinking water supplies, resulting in higher incidences of methemoglobinemia, or blue-baby syndrome, if the water is used for human consumption (Self and Waskom, 2006).

Denitrification results in the loss of nitrate in the form of gaseous emissions of dinitrogen gas (N_2), nitric oxide (NO), and nitrous oxide (N_2O) and is used by wastewater treatment plants to safely reduce nitrate levels in drinking water supplies (Gray, 2004). Urban communities have developed potential "sinks" for nitrate in the form of ponds or green retention basins where denitrification may naturally occur (Zhu et al., 2004). However, the emission of N_2O has become the focus of many recent studies as it represents a potent greenhouse gas with a thermal absorption potential much higher than that of carbon dioxide (Gillenwater, 2002). Modern urban development should incorporate management plans for the offsite movement of DON in regards to water supplies and gaseous emissions.

DISSOLVED ORGANIC PHOSPHORUS

Dissolved organic phosphorus (DOP), like DOC and DON can be a limiting macronutrient to biological production in both terrestrial and aquatic ecosystems. Improperly managed fertilization can lead to the accumulation of phosphorus in the soil profile, potentially increasing the amount of phosphorus found in soil solution (Andraski and Bundy, 2003; King et al., 2000). The fact that DOP easily moves into soil solution decreases its potential for retention in the soil profile and increases the possibility of contamination of aquatic ecosystems (Whalen and Chang, 2001), through offsite movement via efficient urban drainage systems.

Not only is DOP biochemically labile, it also represents a readily available form of phosphorus for microbial metabolism (Orrett and Karl, 1987). DOP has been shown to increase populations of marine microorganisms, specifically phytoplankton and algal communities (Carpenter et al., 1998b), which in turn leads to eutrophication (with the possible occurrence of oxygen depletion) and the potential for die-off of macro-fauna in natural waterways. Metropolitan expansion and the associated offsite consequences of the movement of land applied fertilizer must therefore be effectively understood.

MICROBIAL ECOLOGY

Microbial populations influence ecosystems through their mediation of key nutrient transformations. Carbon and nitrogen mineralization rates in soils have been strongly correlated to microbial biomass (Ingram et al., 2005; Franzluebbers et al., 2000), indicating that microbial populations play an important role in the conversion of

organic forms of carbon and nitrogen, decreasing the availability of DOC and DON. Methane, a much more potent greenhouse gas than carbon dioxide, can be produced from acetate during methanogenesis. Water-logged soils create an anaerobic environment, favorable to methanogenic bacteria, increasing the production of methane (Maier et al., 2000; Xu et al., 2004). Excessive irrigation in an urban setting could potentially lead to increased levels of methane gas. With regards to nitrification, specific species of bacteria, in representative numbers, can also affect N transformation, namely *Nitrosomas* and *Nitrobacter* spp. (Marschner, 2007a). Free-living N_2-fixing bacterial populations can incorporate N into the soil profile (Schippers et al., 1987), increasing the potential for both mineralization and denitrification. The soil microbial biomass also has the ability to immobilize nutrients (Sylvia et al., 1997), in essence making microbial populations a "sink" for nutrients. Understanding the compositional differences of the microbial communities under various types of grasses in the urban landscape could potentially identify possible consequences for over fertilization and irrigation.

OBJECTIVES

The objectives for my research are as follows: 1) To determine the concentrations of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP), as well as other nutrients in the soil solution under three different turfgrasses, and the drainage water from two different sports fields; 2) To identify any seasonal changes in levels of DOC, DON, DOP, and nutrients in soil solution and drainage water; 3) To characterize microbial populations from the native soil and sand-based root zones, with respect to population size, carbon substrate utilization profiles, and community composition; and 4) To identify any seasonal changes in microbial community composition from both a native soil and sand-based root zones.

MATERIALS AND METHODS

RESEARCH SITES

Studies were conducted on the Texas A&M University Campus (West Side), College Station, Texas. Samples were collected from three established turfgrasses; Bermudagrass (*Cynodon dactylon* (L.) Pers), Zoysiagrass (*Zoysia japonica* Steud.), and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze), on a native soil. Two sports fields with sand-based root zones, the Texas A&M University soccer and softball fields were also sampled. The soccer field consists of TifSport Bermudagrass, which is over-seeded in the winter months with a cool season ryegrass (*Lolium perenne* (L.); 3-way blend). The softball field consists of TifWay 419 Bermudagrass and is also over-seeded in the winter months with a ryegrass. Mean annual rainfall and temperature in College Station are 101.0cm and 19.8ºC (Fig. 2) respectively, with mean monthly temperatures ranging from a low of 4.3ºC to a high of 35.3ºC. Soil at all locations was typically slightly acidic with a pH of the soil solution and drainage water samples ranging from 6.2 to 8.8 and 6.8 to 8.5 respectively. The turf plots were located on a Boonville fine sandy loam (fine, smectitic, thermic Chromic Vertic Albaqualfs) formerly under pasture (Wright et al., 2007). Both sports field sand-based root zones were established according to the Airfield® system (Airfield Systems, Edmond, Oklahoma), which consists of washed sod over a 28.0cm (11in) base of sand, placed over a geotextile fabric that allows the downward migration of water and fine particles from the root zone

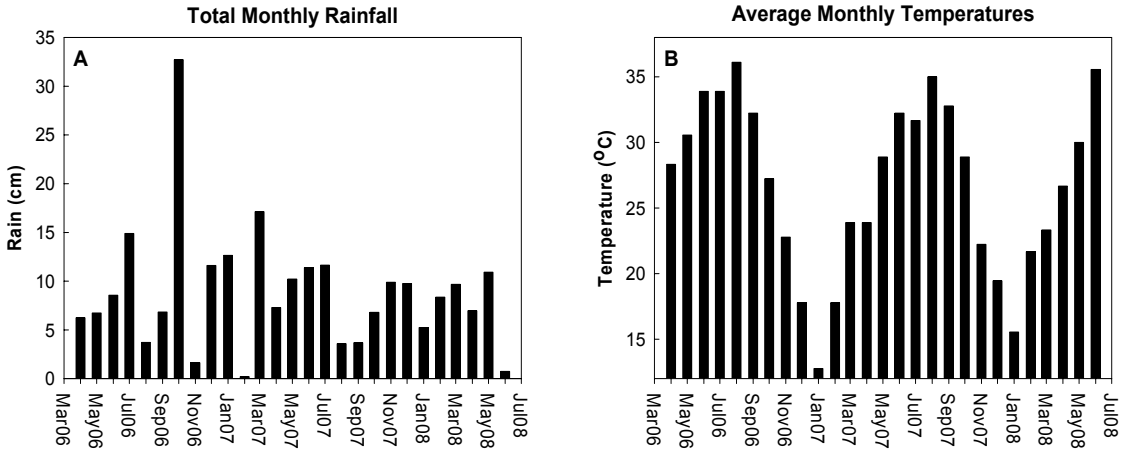

Fig. 2 – Total monthly rainfall (A) and mean temperatures (B) for College Station, Texas during the study period (Weather Underground, 2008).

to a composite grid support structure below. The support structure provides a 2.5cm layer of space that encourages rapid drainage and constant air exchange, and rests on an impervious liner against the sub-grade that slopes to perimeter drains where water is collected and moved away (Fig. 3a). Soil solution samples were collected from the turf plots via slotted PVC wells (3.8cm in diameter) used to collect to a depth of 30cm into the soil (Fig. 3b). Wells were capped with PVC end caps containing minute holes to allow for equilibrium of pressure and to prevent contamination from surface water, grass clippings, etc. The top 5.0cm of slits were sealed with polyester resin to prevent surface-water intrusion into the wells.

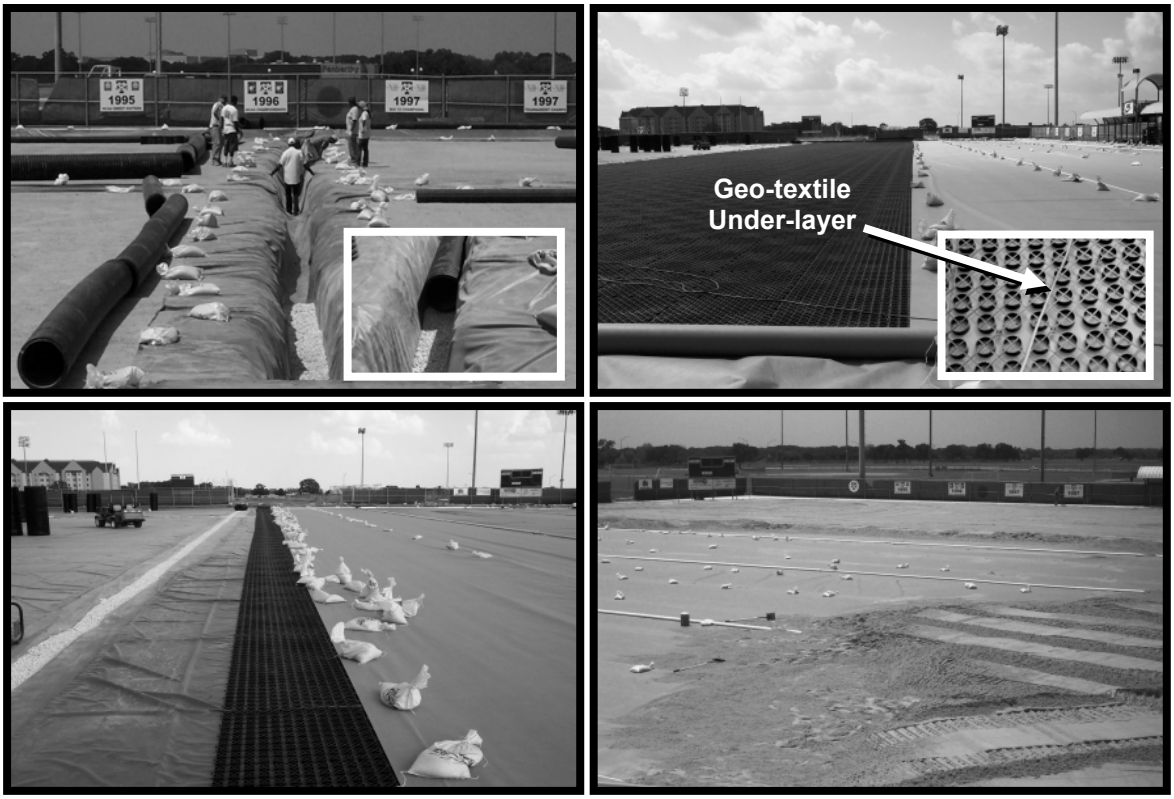

Fig. 3a – Sports field construction. Both sports fields were established according to the Airfield® system (Airfield Systems, Edmond, Oklahoma), with a geo-textile under-layer to aid in field drainage. Soccer field construction is shown below.

SAMPLE COLLECTION

Three PVC wells were placed in each of the three turfgrass plots to act as replicate samples. To retrieve drainage water from the sports fields, the main output drain was used for collection from each field. The sports field drainage systems are

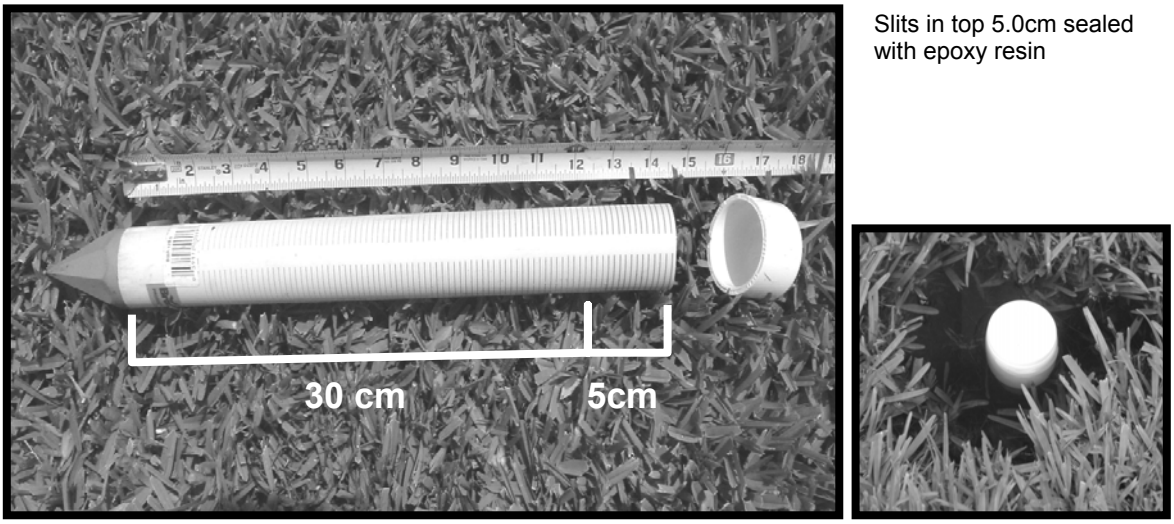

Fig. 3b – PVC wells used for soil solution extraction. Stand pipe well screens, 0.040 well screen slot, 1-1/2". Three wells were placed in each turfgrass plot.

connected underground at a slight gradient that flows to one end of the field and into a single main output drain. Soil solution and drainage samples were collected over a two year period, and immediately stored at 4ºC. Samples were analyzed for DOC within one to two days of collection, while analysis of DON and DOP occurred from 1 week to 4 months after initial collection.

Three arbitrarily selected 2.5-cm diameter core samples (15cm deep) were extracted from three different locations within each of the turfgrass plots and sports fields every three months for a period of one year. Core samples were homogenized from each location, giving a total of fifteen composite samples between the five areas. After collection, samples were immediately analyzed for carbon substrate utilization and

culturable microbial populations, and the remaining portion was stored at -80ºC for subsequent microbial community analyses.

SAMPLE ANALYSIS

Dissolved organic carbon (DOC) in all water samples was quantified using a Total Organic Carbon (TOC) analyzer (Model #700, O.I. Corp., College Station, Texas) per standard operating procedures for a UV-catalyzed persulfate oxidation method. A standard absorption measurement of color (Cuthbert and Giorgio, 1992) was made at 440nm from all samples and was compared with TOC results.

Dissolved organic nitrogen (DON) in all water samples was quantified using a difference method, with total dissolved nitrogen (TDN) determined with a persulfate oxidation method (Qualls et al., 1991) and dissolved inorganic nitrogen (DIN) determined colorimetrically by cadmium reduction to nitrite (Texas AgriLife Extension Soil, Water, and Forage Testing Lab, College Station, TX). The persulfate oxidation method was modified to accommodate smaller sample sizes of 10mL with two replicates each, for a total of 20mL per soil solution or drainage water sample. To account for potential conversion of ammonium to nitrate during storage, samples were tested for levels of ammonium with a catalyzed indophenol method, 1 day to 2 weeks after collection (Chaney and Marbach, 1962).

Dissolved organic phosphorus (DOP) was also quantified using a difference method, with total dissolved phosphorus (TDP) determined by ICP analysis (Texas AgriLife Extension Soil, Water, and Forage Testing Lab, College Station, TX) and

dissolved inorganic phosphorus (DIP) determined using a colorimetric ascorbic acid method (Edwards et al., 1965). Since detergents (which may contain phosphorus) were used to clean some sample bottles, possible residual levels of phosphorus in the sample storage bottles were analyzed. We placed deionized water and tap water into randomly selected sample bottles. The water was then tested a month after initial storage for phosphorus, however, no phosphorus was detected.

Other water chemistry parameters (i.e., electrical conductivity, pH, and other micronutrients) were analyzed by the Texas AgriLife Extension Soil, Water, and Forage Testing Lab, College Station, Texas.

MICROBIAL COMMUNITY ANALYSIS

Microbial population size and composition were determined through three different procedures. Populations of bacteria and fungi were enumerated using standard dilution plate count procedures (Zuberer, 1994). Ten grams of each composite soil or root-zone sample were used to set up a ten-fold dilution series ranging from 10^{-2} to 10^{-6}. Dilutions of 10^{-3} to 10^{-5} were spread plated on Rose-Bengal Streptomycin agar plates (Martin's medium) with three replicates per dilution to enumerate fungal populations. Dilutions of 10^{-5} to 10^{-7} were spread plated on 10% Tryptic Soy Agar (TSA) plates with three replicates per dilution to enumerate bacterial populations.

Microbial carbon source utilization profiles were determined using Biolog® GN2 plates (Biolog, Hayward, California), inoculated with a 10^{-3} dilution from the above dilution series and allowed to incubate for five days at 32ºC. The plates were read at

590nm using a Biolog plate reader at twenty-four hour intervals, with an initial 0 time. Biolog data were analyzed according to standard principal component analysis (PCA) procedures (Garland and Mills, 1991).

A standard fatty acid methyl ester (FAME) method (Cavigelli et al., 1995) was used with the stored (-80ºC) composite samples to determine compositional differences, if any, in the microbial communities associated with the grasses grown on the native soil and sand-based root zones. The FAME profiles were analyzed using PCA in an attempt to identify differences in microbial communities between the various grasses and soil types. FAME profiles were also used to identify changes in composition of microbial communities over the growing season.

CHARACTERIZATION OF DISSOLVED CONSTITUENTS IN SOIL SOLUTIONS AND DRAINAGE WATER FROM WARM-SEASON TURFGRASSES AND SPORTS FIELDS

INTRODUCTION

The encroachment of metropolitan areas into agricultural lands has led to changes in nutrient cycles, which has in turn led to changes in ecosystem composition (McDonnell and Pickett, 1990). Soils that had for generations been subjected to continuous crop production are now being covered with both native and non-native turf species. This is not necessarily a deleterious situation, as turfgrasses have the potential to prevent erosion, improve soil aggregation, provide small-wildlife habitats, improve the aesthetic qualities of the urban environment, and sequester carbon (Beard and Green, 1994; Qian and Follett, 2002). Little is known, however, about the long-term influences of urbanization on organic nutrients, and the resultant influences on carbon (C), nitrogen (N), and phosphorus (P) cycles in urbanized ecosystems. In urban landscapes, organic nutrients can be metabolized by the soil biota, tied up in soil microbial biomass, lost as runoff, leached through the soil profile, etc., just as in agricultural fields. However, agricultural landscapes typically consist of large fields, measured in acres, with generally uniform fertilizer and irrigation rates. Typical urban settings, such as home lawns and sports fields, are generally much smaller and may be subject to extreme variability in fertilization rates and irrigation practices within only a few feet of each other (i.e., neighbors in a residential district fertilize and irrigate at

different rates). The large variability in management practices among the differing types of urban settings makes it difficult to relate prior agricultural soils research on quantities of organic forms of C, N, and P to the growing urban landscape. Therefore, the objective of the study was to determine the levels of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP), as well as other nutrients in the soil solution under three different turfgrasses on a native soil, and in the drainage water from two different sports fields with highly engineered sand-based root zones.

MATERIALS AND METHODS

Studies were conducted at the Texas A&M University Campus (West Side), College Station, Texas. Samples were collected from three established turfgrasses; Bermudagrass (*Cynodon dactylon* (L.) Pers), Zoysiagrass (*Zoysia japonica* Steud.), and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze), on a native soil. The Bermudagrass plot was established in 2001 as part of a compost application study that ended in 2002 (Wright et al., 2007). The Zoysiagrass and St. Augustinegrass plots were established in June of 2002, and they were used in a separate nitrogen fertilization experiment that was conducted in 2007, during our own study (Appendices 1a and 1b list fertilization rates applied to each sub plot). In addition, two sports fields with sand-based root zones; the Texas A&M University soccer and softball fields were also sampled. The soccer field consists of TifSport Bermudagrass and is over-seeded in the winter months with a cool season ryegrass (*Lolium perenne* (L.); 3-way blend). The

softball field consists of TifWay 419 Bermudagrass and is also over-seeded in the winter months with ryegrass (*Lolium perenne* (L.); 3-way blend). The soccer field was established in 2000, while the softball field was established in 2005. Fertilization of the sports fields occurred at the beginning, middle, and end of each sports season (Appendices 2a and 2b), and irrigation for the fields occurred every 8 to 10 days (5.0cm per field) unless there was a rainfall event (Craig Potts, Assistant Manager, Grounds Maintenance, Texas A&M University, personal communication). Clippings were left on the ground at each turf plot and sports field.

Total annual rainfall and mean annual temperature in College Station are 101.0cm and 19.8ºC respectively, and monthly values during the study period are shown in Fig. 2. Mean temperatures ranged from a low of 4.3ºC to a high of 35.3ºC. Soils were typically slightly acidic with the pH of the soil solution and drainage water samples ranging from 6.2 to 8.8 and 6.8 to 8.5 respectively. Higher pH values were observed during the summer months and are likely due to irrigation water (Fig. 4). The turf plots were located on a Boonville fine sandy loam (fine, smectitic, thermic Chromic Vertic Albaqualfs) formerly under pasture (Wright et al., 2007). Both sports-field sand-based root zones were established according to the Airfield® system (Airfield Systems, Edmond, Oklahoma), washed sod was laid over a base of pure sand 28.0cm deep with a geo-textile under-layer to aid in field drainage (Fig. 3a).

Soil solution samples were collected from June 2006 through June 2008, from the turf plots via slotted PVC wells (3.8cm in diameter) placed approximately 30.0cm deep into the soil (Fig. 3b). Wells were capped to prevent contamination from surface

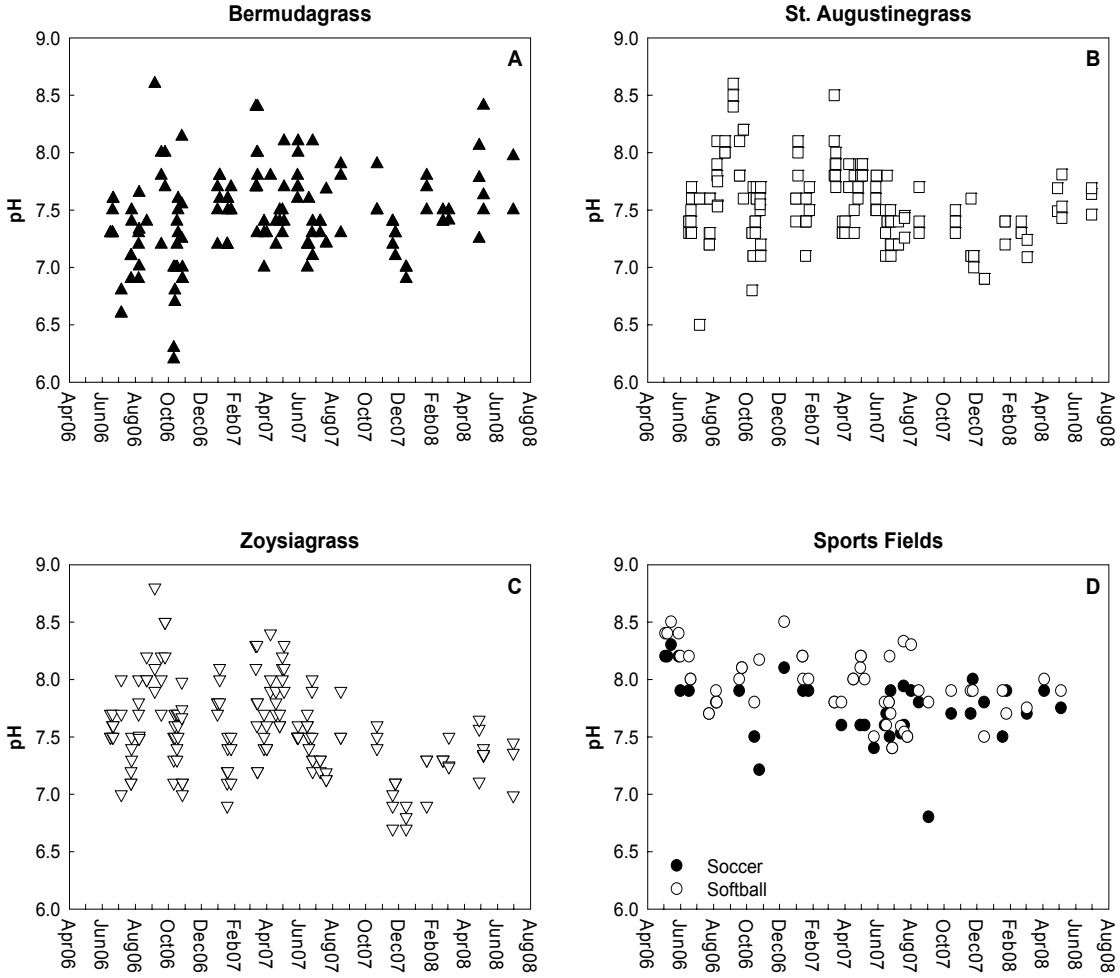

Fig. 4 – pH values for soil solution and drainage water samples. Soil solution was collected from underneath three turfgrasses – Bermudagrass (A), St. Augustinegrass (B), and Zoysiagrass (C), and drainage water samples collected from two sports fields – Soccer and Softball (D). The individual sample values are plotted for each date.

water, grass clippings, etc. The top 5.0cm of slits were sealed with polyester resin to prevent surface water intrusion into the wells. Three PVC wells (Fig. 5) were placed in each of the three turf grass plots to act as replicate samples. Wells for the Bermudagrass plot were placed in the "control" (non-composted) areas of the original compost application study. Drainage water samples were collected from May 2006 through May 2008, from both the soccer and softball fields. To retrieve drainage water from the sports fields, the single main output drain line was used for collection at each site (Fig. 5). Each sports field has a highly engineered drainage system (Airfield® system) that is located underneath the 28.0-cm layer of sand. The sand and drainage system are separated by a geotextile/fabric layer, which retains water in the soil and allows for slow percolation of excess water into the drain pipes (Fig. 3a). Water then flows to one end of the field, and out through a single main output drain. Soil solution and drainage samples were stored at 4ºC within thirty minutes after collection. Samples were analyzed for DOC, bicinchoninic acid (BCA)/reactive carbon, and ammonium within one to two days of collection. Analysis of DON, DOP, and absorbance at 440nm occurred from 1 week to 4 months after initial collection.

Six soil core samples (2.5-cm in diameter and 15.0-cm deep) were collected from each location for determination of soil organic C and N (Table 1) in October 2007. All soil samples were sieved through a 2.0-mm screen to facilitate the removal of plant materials (i.e., roots, leaves, etc.) and subsequently the homogenized soil samples were analyzed by the Texas AgriLife Extension Soil, Water, and Forage Testing Lab, College Station, TX. Rainfall was also collected at the turf plot location, using a collection

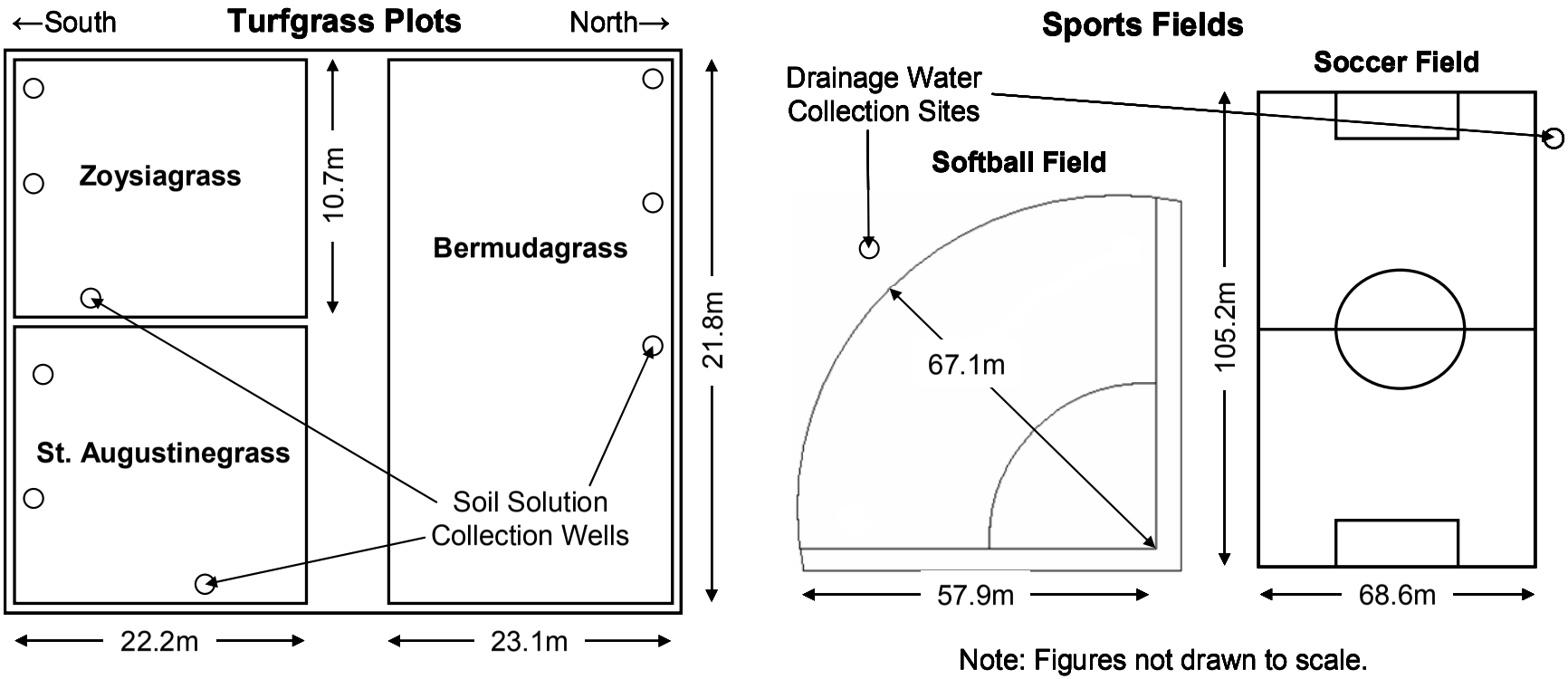

Fig. 5 – Well placement and drainage water collection. Three wells were placed in each individual turf plot for soil solution collection. The single main output drain line for each sports field was used for collection of drainage water.

Table 1 – Soil carbon (C) and nitrogen (N) contents of three turfgrass plots (Bermudagrass, Zoysiagrass, and St. Augustinegrass) and two sports fields (soccer and softball).

Sampling Location	Organic C (%)	Organic Matter (%)*	Total N (%)	C:N
Bermudagrass Plot	1.84	3.17	0.17	10.56
St. Augustinegrass Plot	0.87	1.50	0.11	7.60
Zoysiagrass Plot	1.28	2.20	0.12	9.84
Soccer Field	0.39	0.67	0.06	8.30
Softball Field	0.27	0.47	0.05	5.03

*%OM = Organic C (%) x 1.723

device consisting of a funnel, collection bottle, support structure, and fine plastic mesh to prevent contamination from insects and wildlife entering into the collection bottle.

Dissolved organic carbon in all water samples was quantified using a TOC analyzer (Model #700, O.I. Corp., College Station, TX) per standard operating procedures with a UV-catalyzed persulfate oxidation method. A standard absorbance measurement of color (OD = 440nm) was determined for all samples and was used to compare results with the auto-analyzer (Cuthbert and Giorgio, 1992). A bicinchoninic acid (BCA) assay was also used to identify a subfraction of the DOC. The BCA reagent can, however, react with proteins, carbohydrates, and other low-molecular weight

dissolved organics, such as amino acids and phenolic compounds (Keil and Kirchman, 1993; Smith et al., 1985).

Dissolved organic nitrogen levels were quantified using a difference method, with total dissolved nitrogen (TDN) determined by a persulfate oxidation method (Qualls et al., 1991) and dissolved inorganic nitrogen (DIN) determined by a ICP spectrophotometry (Texas AgriLife Extension Soil, Water, and Forage Testing Lab, College Station, TX). The persulfate oxidation method was modified to accommodate smaller sample sizes of 10.0mL with two replicates each, for a total of 20.0mL per sample. A standard curve was established with each run to verify accuracy and adjust measurements accordingly. To account for potential conversion of ammonium to nitrate during storage, samples were tested for levels of ammonium with a catalyzed indophenol method (Chaney and Marbach, 1962), 1 day to 2 weeks after collection.

Dissolved organic phosphorus levels were also quantified using a difference method, with total dissolved phosphorus (TDP) determined by an ICP analysis (Texas

AgriLife Extension Soil, Water, and Forage Testing Lab) and dissolved inorganic phosphorus (DIP) determined using an ascorbic acid method (Edwards et al., 1965). Since sample bottles were occasionally cleaned with detergent and reused, we also attempted to check for organic phosphorus contamination inside the sample storage bottles. This was achieved by adding deionized water and distilled water into selected sample bottles, holding them for one month, and analyzing the water for organic phosphorus.

Additional analyses of other water chemistry parameters (i.e., electrical conductivity, pH, and other nutrients such as iron, zinc, sulfur, etc.) were performed by the Texas AgriLife Extension Soil, Water, and Forage Testing Lab.

Linear regression and one-way ANOVA (Kruskal-Wallis Test) analyses were performed with SigmaStat® 3.5 (Systat Software, San Jose, California). Logarithmic transformations were calculated, when indicated, as $\log_{10}(X + 1)$, where "X" is the original data value, to achieve normality of the data.

RESULTS

Concentrations of DOC (Table 2) in the soil solution samples from the turf plots ranged from 9.3mg L^{-1} to 135.3mg L^{-1}, with a mean of 61.2mg L^{-1} ± 22.2. DOC concentrations in the drainage water samples from the sports fields ranged from 1.8mg L^{-1} to 118.4mg L^{-1}, with a mean of 22.4mg L^{-1} ± 19.6. The Zoysiagrass plot typically had the highest levels of DOC of the three turfgrasses on the Booneville soil throughout the study with a mean of 65.4mg L^{-1} ± 20.0, while the soccer field tended to have higher concentrations of DOC (mean of 25.1mg L^{-1} ± 21.5) than the softball field.

The soil solution samples from the turfgrasses on the native soil and drainage water samples from sports fields on sand-based root zones both demonstrated seasonal trends in levels of DOC (Fig. 6). Peak levels of DOC in both the turf plots and sports fields occurred in late July to August of both 2006 and 2007, with decreasing levels observed during the winter months. The growing season in College Station begins between March and April and reaches a peak around June or July, which may explain the higher levels of DOC observed towards the end of summer.

Dissolved organic nitrogen (DON) concentrations (Table 2) in the soil solution samples from the turfgrass plots ranged from 0.0mg L^{-1} to 43.5mg L^{-1}, with a mean of 5.6mg L^{-1} ± 3.5. Concentrations of DON in the drainage water from the sports fields ranged from 0.0mg L^{-1} (non-detectable) to 58.5mg L^{-1}, with a mean of 6.3mg L^{-1} ± 8.0. The St. Augustinegrass soil solution samples had the highest mean concentrations of DON among the turfgrass plot samples, but also the greatest amount of variability (6.1mg L^{-1} ± 5.0). The soccer field drainage water samples tended to have higher mean

Table 2 - Mean concentrations and range of values for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP) for each sampling location. Mean concentrations are given as the mean value plus or minus the standard deviation. Letters indicate signficant differences (Kruskal-Wallis one-way ANOVA, $p < 0.05$). "n" represents the number of samples analyzed.

Sampling Location	Mean DOC mg L^{-1}	DOC Range mg L^{-1}	Mean DON mg L^{-1}	DON Range mg L^{-1}	Mean DOP mg L^{-1}	DOP Range mg L^{-1}
Bermudagrass Plot	**52.2 ± 21.7^{b}** (n = 135)	**9.3 - 135.3**	**5.5 ± 2.7^{d}** (n = 78)	**0.7 - 12.6**	**1.3 ± 1.9^{e}** (n = 83)	**ND - 8.0**
St. Augustinegrass Plot	**64.8 ± 22.6^{a}** (n = 152)	**22.0 - 128.9**	**6.1 ± 5.0^{d}** (n = 88)	**ND* - 43.5**	**1.3 ± 2.4^{ef}** (n = 92)	**ND - 11.6**
Zoysiagrass Plot	**65.4 ± 20.0^{a}** (n = 156)	**19.6 - 117.6**	**5.3 ± 2.1^{d}** (n = 96)	**ND - 11.9**	**0.6 ± 1.3^{ef}** (n = 96)	**ND - 6.2**
Soccer Field	**25.1 ± 21.5^{c}** (n = 47)	**3.9 - 118.4**	**7.1 ± 9.5^{d}** (n = 47)	**ND - 58.5**	**0.1 ± 0.1^{f}** (n = 47)	**ND - 0.4**
Softball Field	**19.8 ± 17.3^{c}** (n = 47)	**1.8 - 87.3**	**5.5 ± 6.1^{d}** (n = 47)	**ND - 35.6**	**0.5 ± 1.7^{ef}** (n = 47)	**ND - 8.3**

*ND - non-detectable

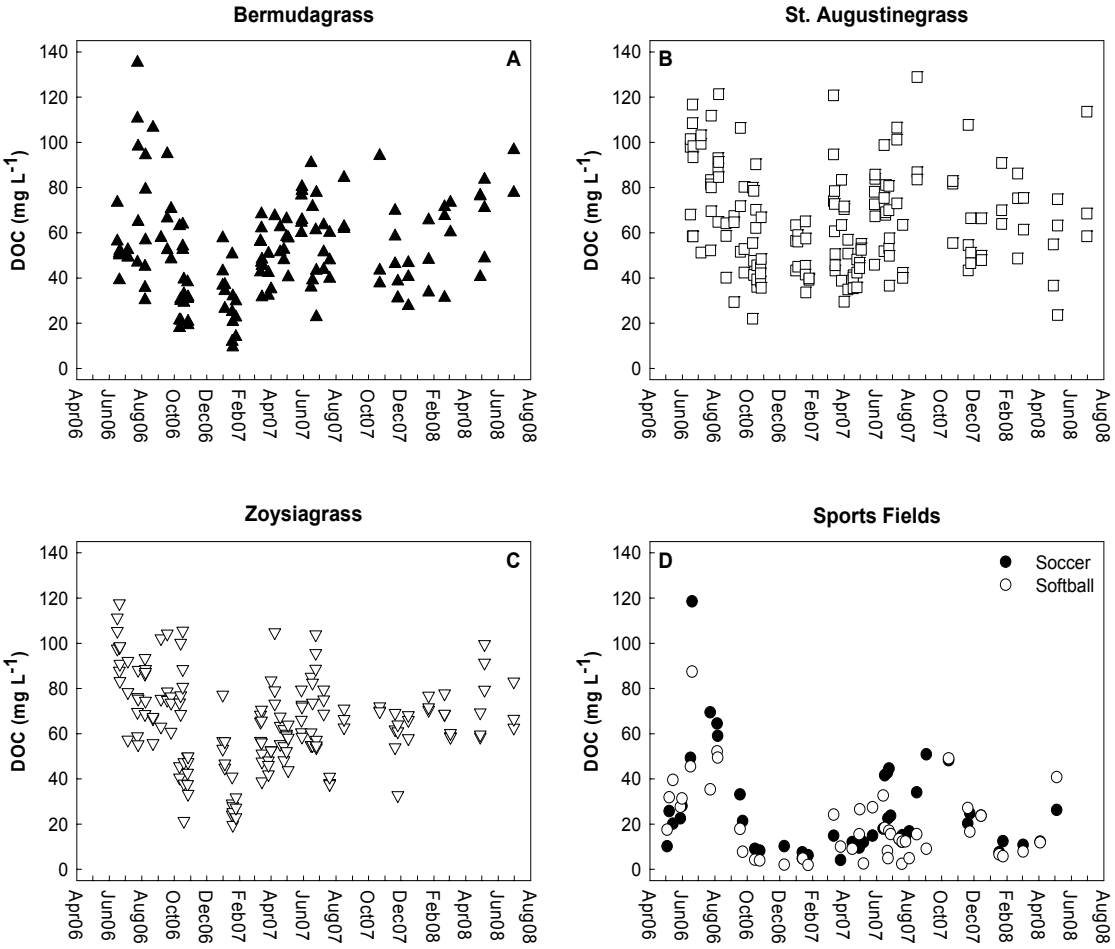

Fig. 6 – Dissolved organic carbon (DOC) in soil solutions and drainage water samples. DOC concentrations for soil solutions from the Bermudagrass (A), St. Augustinegrass (B), and Zoysiagrass (C) plots and drainage water from the soccer and softball (D) fields. The individual sample values are plotted for each date.

concentrations of DON, in addition to higher variability, (7.1mg L^{-1} ± 9.5) than the softball field (5.5 ± 6.1), as indicated by the larger standard deviation.

The turfgrasses on the Boonville soil and sports fields on sand-based root zones also showed seasonal trends in concentrations of DON, with regards to the soil solution and drainage water samples (Fig. 7). Peak values of DON were observed in the turf plots and sports fields during August and September of each year, with a decline during the winter months. The DON concentrations were fairly uniform across the three turfgrasses, which is interesting because the DON values in the Zoysiagrass and St. Augustinegrass plots included in the concurrent fertilization study did not appear to have been influenced by the fertilization. The concentrations of DON observed for the sports fields varied between the soccer and softball fields, with the highest values predominantly found in the drainage water from the soccer field. This may reflect a difference in fertilizer applications.

The potential for conversion of ammonium to nitrate during long-term storage was determined by measuring residual levels of ammonium. We observed little to no ammonium in any of the samples. This indicates there was little potential for conversion to nitrate over a prolonged storage time period, and as such, storage time should not influence our DON measurements, except if samples are instead being mineralized. We retested several samples stored for a year and found the difference in

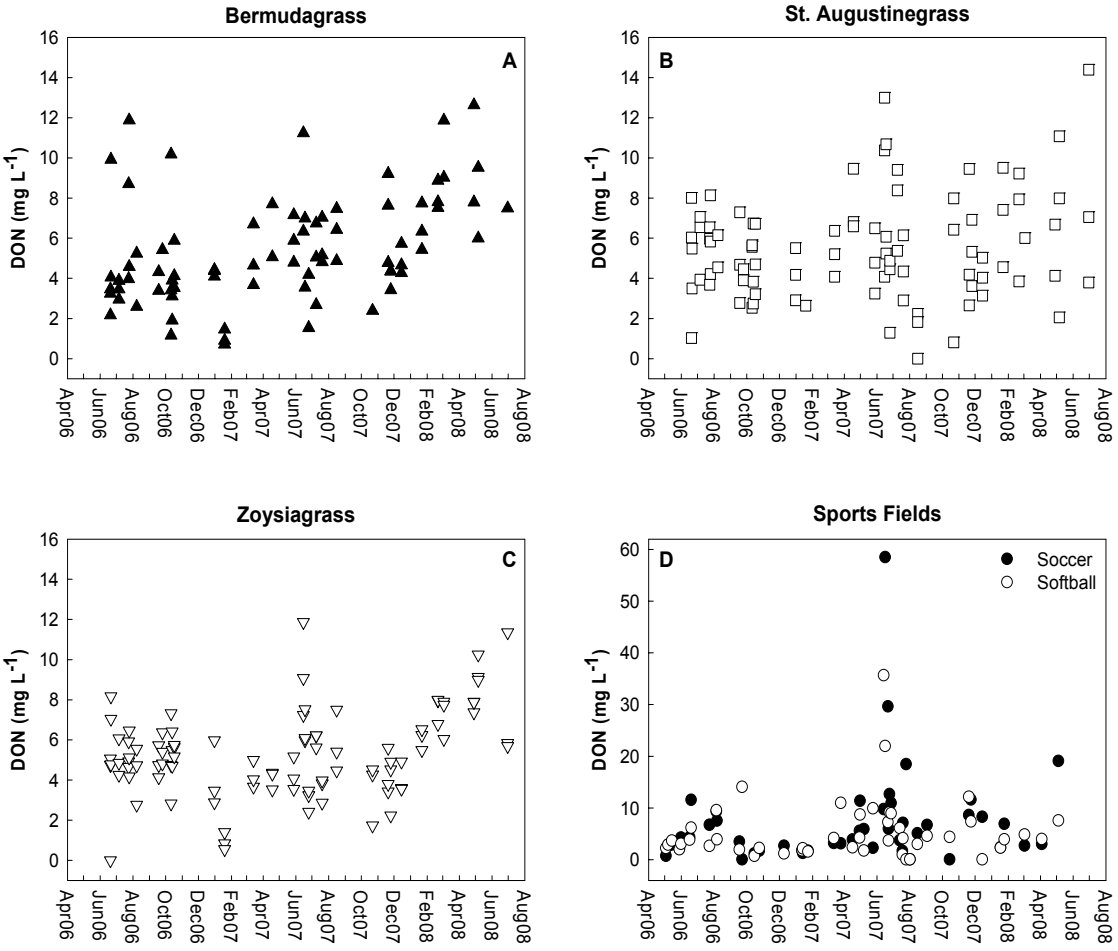

Fig. 7 – Dissolved organic nitrogen (DON) in soil solutions and drainage water samples. DON concentrations for soil solutions from the Zoysiagrass (A), Bermudagrass (B), and St. Augustinegrass (C) plots and drainage water from the soccer and softball (D) fields. The individual sample values are plotted for each date. Note the change in y-axis scaling for the sports fields (D).

DON values to be approximately 1.05mg L^{-1} ± 1.38 (n = 8), indicating a negligible change (average of 21% change) in our original values.

We observed similar seasonal changes in concentrations of DOC and DON, and we next attempted to determine the relationship between DOC and DON, as well as other parameters (Figs. 8-10). However, regressing DOC on DON yielded a weak correlation in both soils, but there was still a positive trend (Fig. 8), with a mean DOC:DON ratio ranging from 4.9:1 in the sand-based root zones to 13.4:1 in the native soil (Table 3). When the data for the individual grasses in the turf plots were log transformed and compared, we observed a stronger correlation between DOC and DON in the Bermudagrass plot ($r^2 = 0.518$, $p < 0.001$) and the Zoysiagrass plot ($r^2 = 0.279$, $p < 0.001$), than in the St. Augustinegrass plot ($r^2 = 0.207$, $p < 0.001$). The correlation of DOC with DON was slightly stronger for the soccer field ($r^2 = 0.179$, $p = 0.003$) than it was for the softball field ($r^2 = 0.168$, $p = 0.004$).

Color ($Abs._{440nm}$) was highly correlated with DOC in soil solutions and drainage water from the sports fields, regardless of soil type (Fig. 9). In general, the more highly colored solutions (greater absorbance at 440nm) were obtained from the turf plots, corresponding to their higher levels of DOC (Table 3). During the late summer to early fall, some of the samples had the appearance of strongly brewed tea.

Bicinchoninic acid (BCA)-reactive C in soil solution and drainage water samples were also highly correlated with DOC levels, with a mean BCA:DOC ranging from

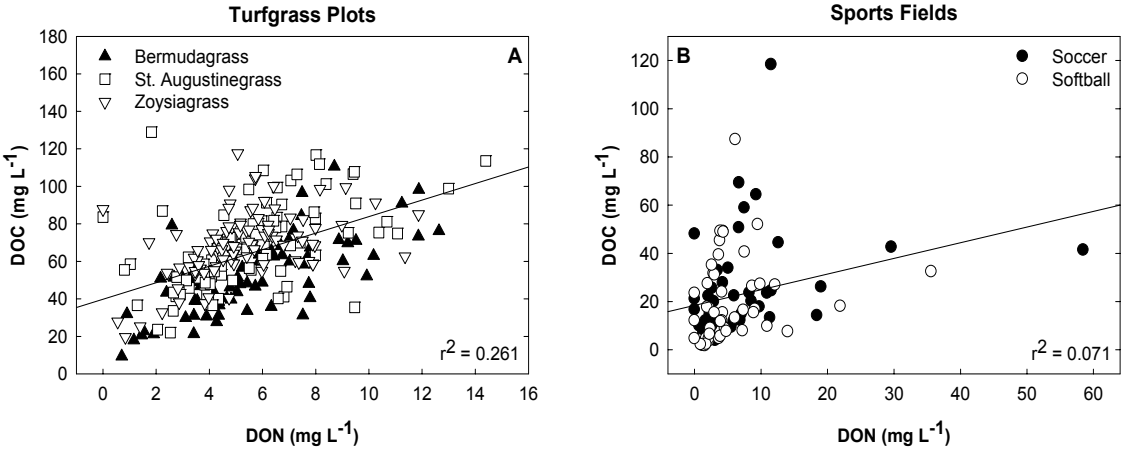

Fig. 8 – The relationship between dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in soil solution samples from three turfgrass plots (A) and drainage water samples from two sports fields (B).

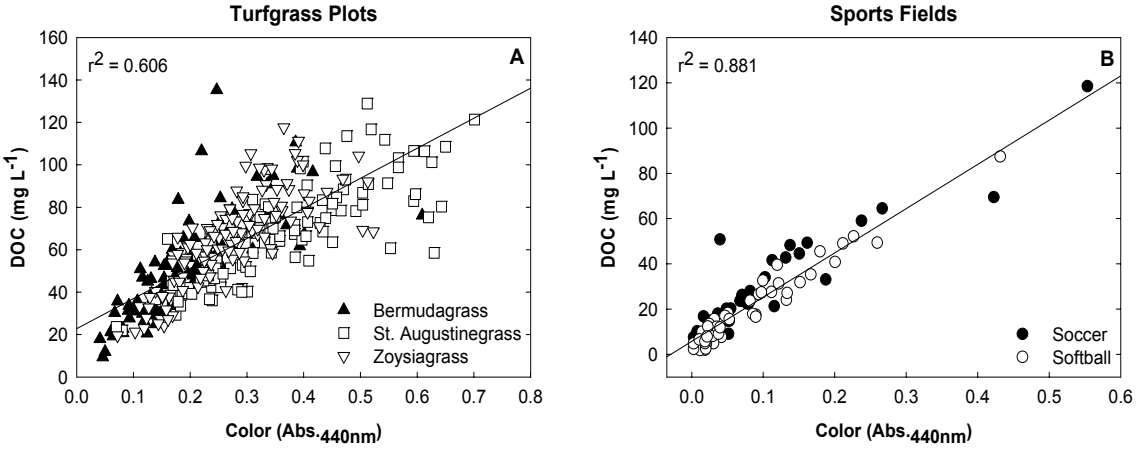

Fig. 9 – The relationship of dissolved organic carbon (DOC) with color ($Abs._{440nm}$) for soil solution samples from three turfgrass plots (A) and drainage water samples from two sports fields (B).

Table 3 - Comparisons of dissolved organic carbon (DOC) to: dissolved organic nitrogen (DON); color ($Abs._{440nm}$); and a bicinchoninic acid (BCA) assay. Correlations for each comparison are given as the r^2 value. "n" represents the number of samples analyzed. Kruskal-Wallis one-way ANOVA was used to determine p-values.

Sampling Location	DOC:DON Mean	Correlation DOC:DON (r^2)*	Color ($Abs._{440nm}$) to DOC Mean	Correlation Color to DOC (r^2)	%BCA-C of DOC Mean**	Correlation BCA-C to DOC (r^2)
Bermudagrass Plot	**11.1:1 ± 5.1** (n = 78)	**0.518** (p < 0.001)	**0.4 ± 0.1** (n = 106)	**0.594** (p < 0.001)	**11.4 ± 4.3** (n = 135)	**0.478** (p < 0.001)
St. Augustinegrass Plot	**14.4:1 ± 10.7** (n = 88)	**0.207** (p < 0.001)	**0.5 ± 0.1** (n = 118)	**0.667** (p < 0.001)	**12.2 ± 3.7** (n = 152)	**0.652** (p < 0.001)
Zoysiagrass Plot	**14.4:1 ± 6.1** (n = 96)	**0.279** (p < 0.001)	**0.4 ± 0.1** (n = 125)	**0.619** (p < 0.001)	**11.6 ± 3.4** (n = 156)	**0.583** (p < 0.001)
Soccer Field	**4.9:1 ± 3.4** (n = 47)	**0.179** (p = 0.003)	**0.3 ± 0.1** (n = 47)	**0.859** (p < 0.001)	**8.3 ± 3.0** (n = 47)	**0.891** (p < 0.001)
Softball Field	**4.8:1 ± 4.3** (n = 47)	**0.168** (p = 0.004)	**0.4 ± 0.2** (n = 47)	**0.942** (p < 0.001)	**10.3 ± 3.4** (n = 47)	**0.925** (p < 0.001)

*Log transformed data ($\log_{10} + 1$)

**Percentage of DOC that reacts with BCA reagent.

9.7% in the sand-based soil to 11.7% in the native soil (Fig. 10). The drainage water from the sports fields showed a stronger correlation between BCA and DOC (Table 3), and this may be due in part to the lower levels of organics found in the sand-based root zone.

Dissolved organic phosphorus (DOP) concentrations (Table 2) in the soil solutions from the turfgrass plots ranged from 0.0mg L^{-1} (non-detectable) to 11.6mg L^{-1}, with a mean of 1.1mg L^{-1} ± 1.9. The values of DOP in the drainage water samples from the sports fields ranged from 0.0mg L^{-1} to 8.3mg L^{-1}, with a mean of 0.3mg L^{-1} ± 1.2. The Bermudagrass soil solutions had the highest mean concentration (1.3mg L^{-1} ± 1.9) among the turfgrass plots. The softball field drainage water samples had the highest mean amount of DOP (0.5mg L^{-1} ± 1.7), as well as the greatest amount of variability among the sports fields.

Concentrations of DOP fluctuated throughout the year (Fig. 11), with a noticeable rise during the end of the study in the soil solutions from the turfgrass plots. However, drainage water from the sports fields contained little DOP with the exception of the higher values in the summer of 2006 and December 2007. Total phosphorus (TP) amounts (Data not shown) also had similar seasonal trends, and appeared to be predominantly made up of DOP. Highest concentrations of DOP were observed in the turf plots, with a peak of 11.6mg L^{-1} in the St. Augustinegrass plot. The Bermudagrass tended to have higher levels of DOP than the other turfgrasses earlier in the study, but

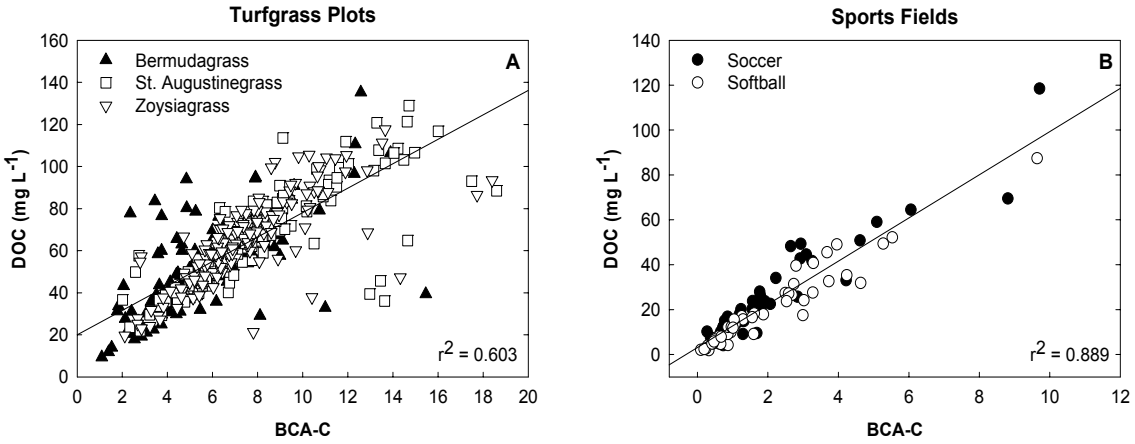

Fig. 10 – The relationship of dissolved organic carbon (DOC) to bicinchoninic acid (BCA)-reactive carbon in soil solution samples from three turfgrass plots (A) and drainage water samples from two sports fields (B).

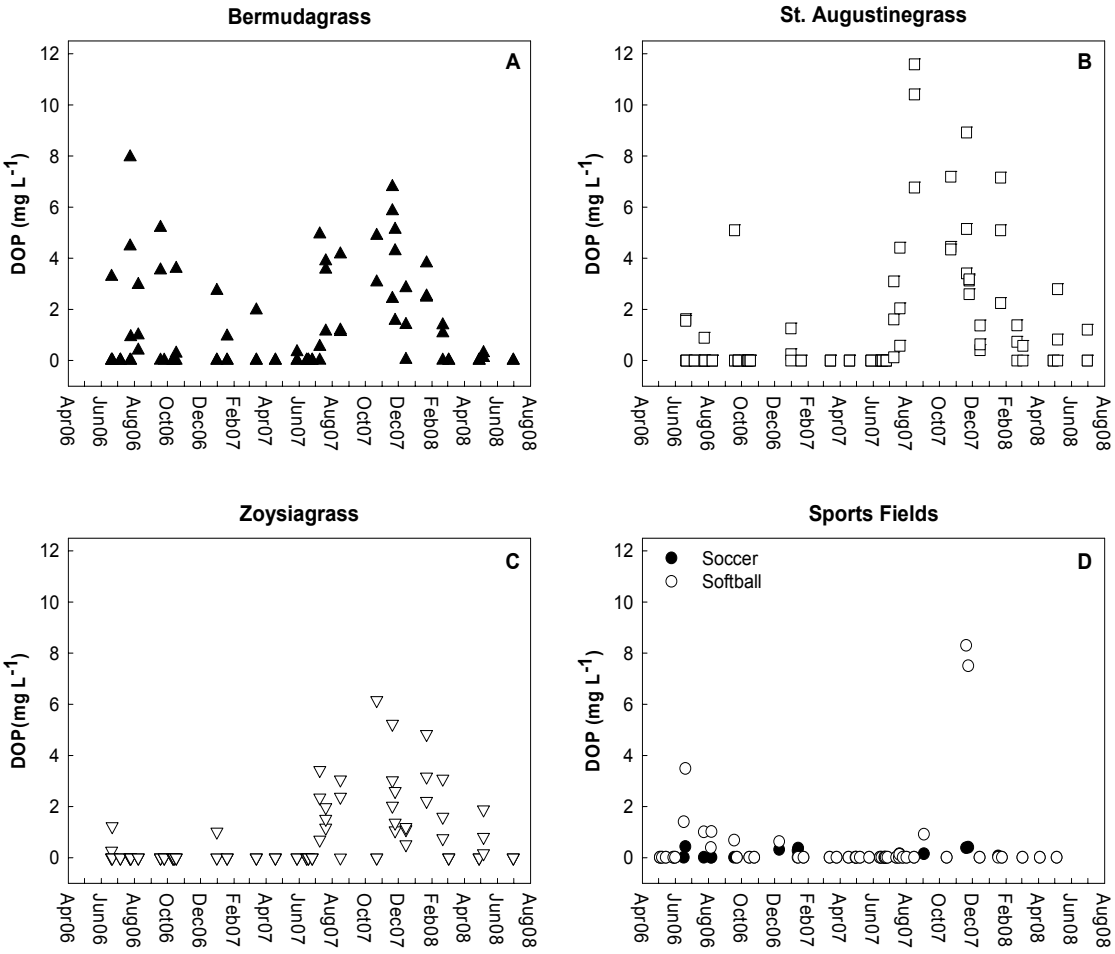

Fig. 11 - Dissolved organic phosphorus (DOP) in soil solutions and drainage water samples. DOP concentrations of soil solutions from the Bermudagrass (A), St. Augustinegrass (B), and Zoysiagrass (C) plots and drainage water from the soccer and softball (D) fields. The individual sample values are plotted for each date.

towards the end of 2007, highest concentrations were observed in the St. Augustinegrass plot. Concentrations of DOP in the drainage water samples were generally lower than those of the soil solutions, with a peak of 8.3mg L^{-1} in the drainage from the softball field in July of 2006. Highest concentrations of DOP in the drainage water samples were from the softball field, with values of DOP for the soccer field never exceeding 1.0mg L^{-1} for any collection date.

Dissolved inorganic nitrogen (DIN) concentrations (Table 4) in soil solutions from the turfgrass plots ranged from 0.0mg L^{-1} (non-detectable) to 24.9mg L^{-1}, with a mean of 1.1mg L^{-1} ± 2.9. Concentrations of DIN in the drainage water samples from the sports fields ranged from 0.0mg L^{-1} to 57.3mg L^{-1}, with a mean of 7.4mg L^{-1} ± 10.8. For our purposes, DIN was considered to be principally nitrate, since we did not observe significant ammonium in any samples (data not shown). The Bermudagrass plot soil solutions had the highest mean values for DIN (1.6 ± 2.8) among the turfgrass plots. The drainage water samples from the soccer field had the highest concentrations of DIN (7.6 ± 11.6), but also showed the greatest amount of variability.

Seasonal concentrations of DIN in soil solutions and drainage water samples are shown in Fig. 12. There were seasonal variations in both the turfgrass plots and sports fields, with peak levels occurring in 2007 during April and May for the turf plots, and June and July for the sports fields. There was twice as much DIN in the drainage water samples from the sports fields, with a peak of 57.3mg L^{-1}, as compared to the turf plots, with a peak of 24.9mg L^{-1}. The nitrogen fertilization experiment, conducted during the

Table 4 – Mean concentrations and range of values for dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) for each sampling location. Mean concentrations are given as the mean value plus or minus the standard deviation.

Sampling Location	Mean DIN mg L^{-1}	DIN Range mg L^{-1}	Mean DIP mg L^{-1}	DIP Range mg L^{-1}
Bermudagrass Plot	**1.6 ± 2.8** (n = 135)	**0.0 - 19.8**	**5.1 ± 2.5** (n = 83)	**1.6 - 12.1**
St. Augustinegrass Plot	**1.6 ± 3.9** (n = 152)	**0.0 - 24.9**	**5.7 ± 2.6** (n = 92)	**1.5 - 14.1**
Zoysiagrass Plot	**0.4 ± 1.0** (n = 156)	**0.0 - 10.3**	**4.8 ± 1.8** (n = 96)	**1.2 - 10.2**
Soccer Field	**7.6 ± 11.6** (n = 47)	**0.0 - 57.3**	**1.1 ± 0.6** (n = 47)	**0.3 - 2.9**
Softball Field	**7.1 ± 10.0** (n = 47)	**0.0 - 39.9**	**1.9 ± 1.3** (n = 47)	**0.6 - 6.0**

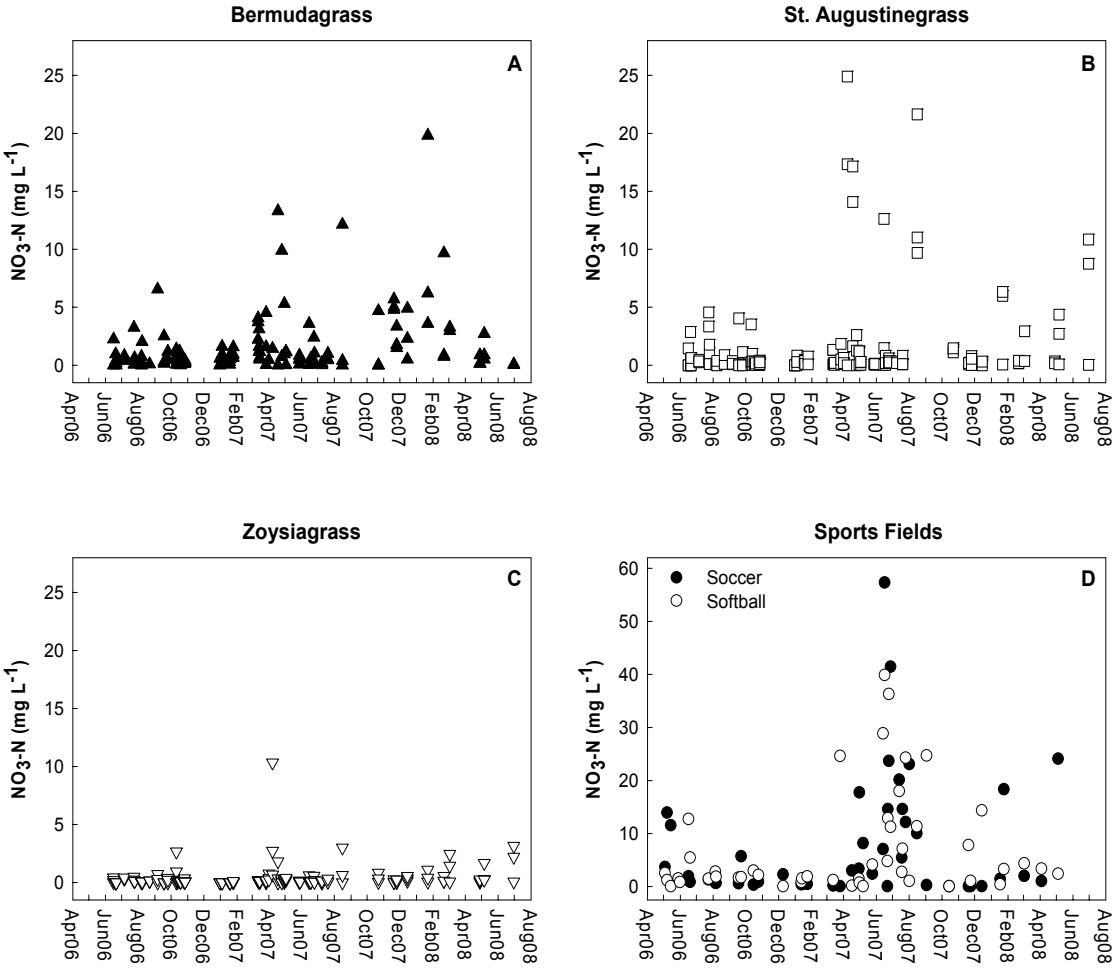

Fig. 12 - Dissolved inorganic nitrogen (DIN) in soil solutions and drainage water samples. DIN concentrations for soil solutions from the Bermudagrass (A), St. Augustinegrass (B), and Zoysiagrass (C) plots and drainage water from the soccer and softball (D) fields. The individual sample values are plotted for each date. Note the change in y-axis scaling for the sports fields (D).

study, did appear to influence the St. Augustine plot, as indicated by the elevated levels of DIN observed during April and May of 2007. Interestingly, the DIN in the soil solution from the Zoysiagrass plot did not show as marked an increase (other than a peak in April of 10.3mg L^{-1}) in the fall of 2007 as did the soil solutions from the St. Augustinegrass and Bermudagrass plots.

I next attempted to identify the causes of the high concentrations of both DIN and DON observed in 2007, by comparing the results to the reported applied nitrogen (N) fertilizer (Fig. 13). Peak concentrations of DIN and DON appeared to coincide with fertilization events, however, this was not true in all cases. The soccer field did not show an increase in DIN or DON after one fertilizer application, and in another instance the softball field had an increase in both DIN and DON when no fertilizer was reported to have been applied. Other than those two events, it was clear that fertilizer applications influenced concentrations of both DIN and DON.

Dissolved inorganic phosphorus (DIP) concentrations (Table 4) in the soil solutions from the turfgrass plots ranged from 1.2mg L^{-1} to 14.1mg L^{-1}, with a mean of 5.2mg L^{-1} ± 2.3. Concentrations of DIP in the drainage water samples from the sports fields ranged from 0.3mg L^{-1} to 6.0mg L^{-1}, with a mean of 1.5mg L^{-1} ± 1.1. The soil solutions from the St. Augustinegrass plot had the highest concentrations of DIP (5.7mg L^{-1} ± 2.6) among the turfgrass plots, while the drainage water samples from the softball field had the highest amounts of DIP (1.9mg L^{-1} ± 1.3) among the sports fields.

Concentrations of DIP (Fig. 14) were greater in the soil solutions from the native soil, than in the drainage water from the sand-based root zones. Seasonal variations in

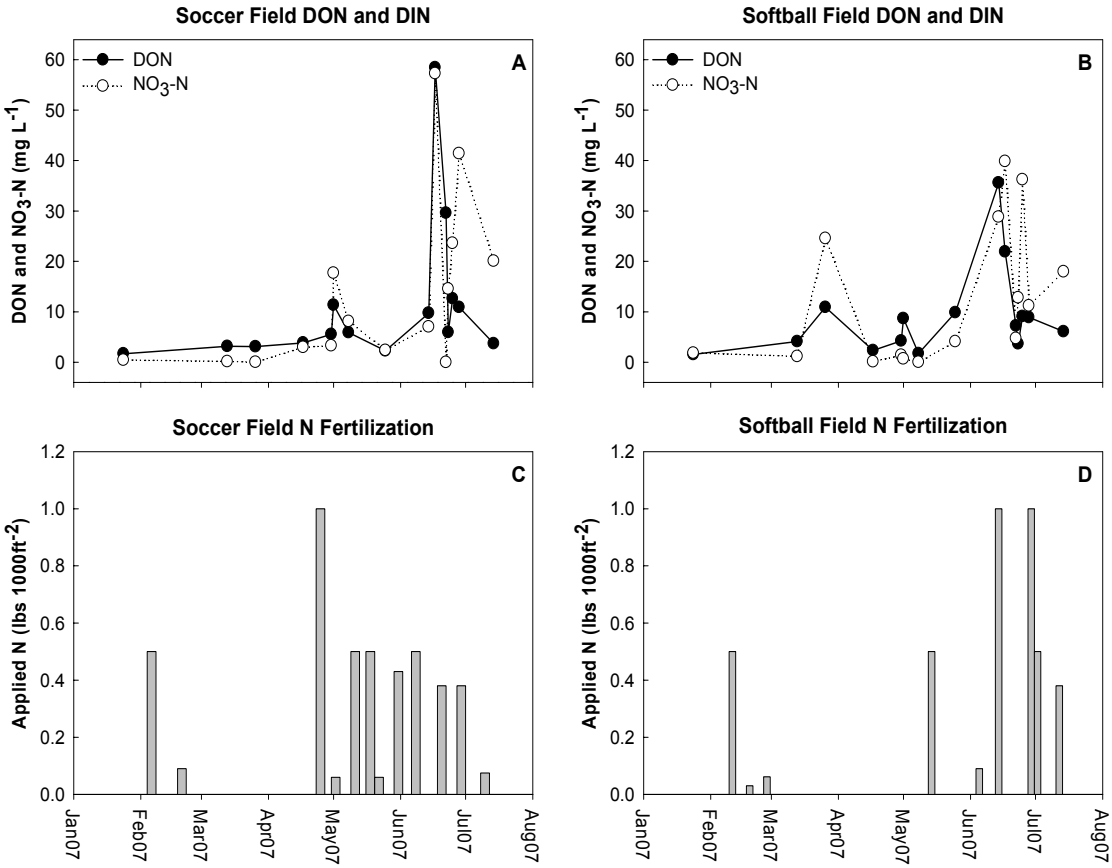

Fig. 13 – Comparison of dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN) for the soccer field (A) and softball field (B), to applied nitrogen (N) fertilizer for the soccer field (C) and softball field (D) in 2007. NO_3-N is considered equivalent to DIN.

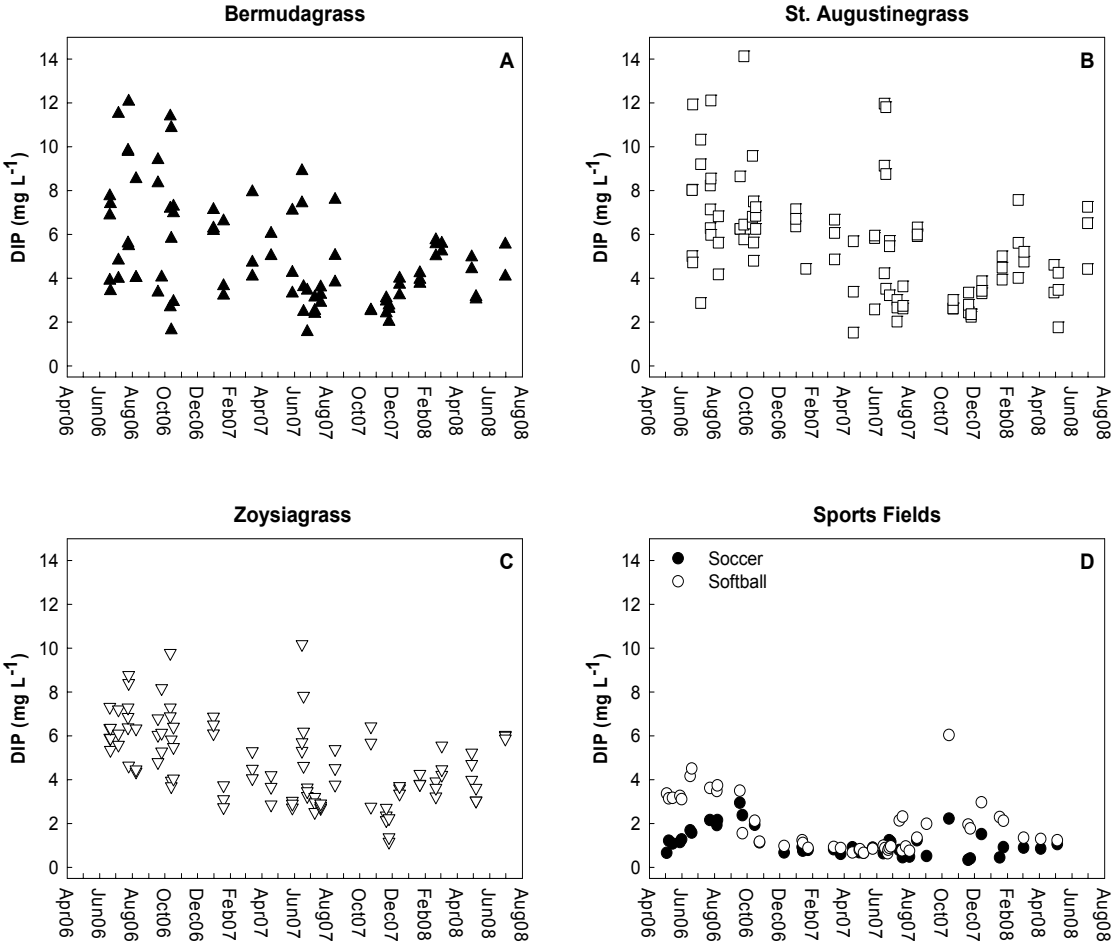

Fig. 14 - Dissolved inorganic phosphorus (DIP) in soil solutions and drainage water samples. DIP concentrations for soil solutions from the Bermudagrass (A), St. Augustinegrass (B), and Zoysiagrass (C) plots and drainage water from the soccer and softball (D) fields. The individual sample values are plotted for each date.

the amount of DIP in the native soil were apparent, with elevated concentrations observed in the mid-summer through mid-fall. The sand-based sports fields displayed a similar seasonal pattern, but the peak DIP values appeared later in 2007. Peak values of DIP ranged from 6.0mg L^{-1} in the sports fields to 14.1mg L^{-1} in the turf plots. The Bermudagrass plot appeared to have the highest concentrations of DIP among the turf plots throughout the study. Drainage water from the softball field tended to have greater concentrations of DIP than drainage from the soccer field.

The sodium (Na) contents and electrical conductivity from the soil solutions and drainage water samples were highly correlated (Fig. 15), but no other strong associations among other micronutrients were apparent (data not shown). Municipal water was used for irrigation at the turfgrass plots and sports fields, and since College Station, TX has high concentrations of Na in the tap water (up to 200mg L^{-1}, City of College Station, 2006) this leads to the relatively high amounts of Na observed throughout irrigated periods of the study. This is evidenced by peak concentrations occurring during frequently irrigated time periods (Fig. 16). The St. Augustine plot typically had the highest Na levels among the turf plots, with a peak of 511mg L^{-1}. The soccer field reached a peak level of 360mg L^{-1}, but was not consistently higher than the softball field.

We also collected and analyzed rain water, as a potential source of added

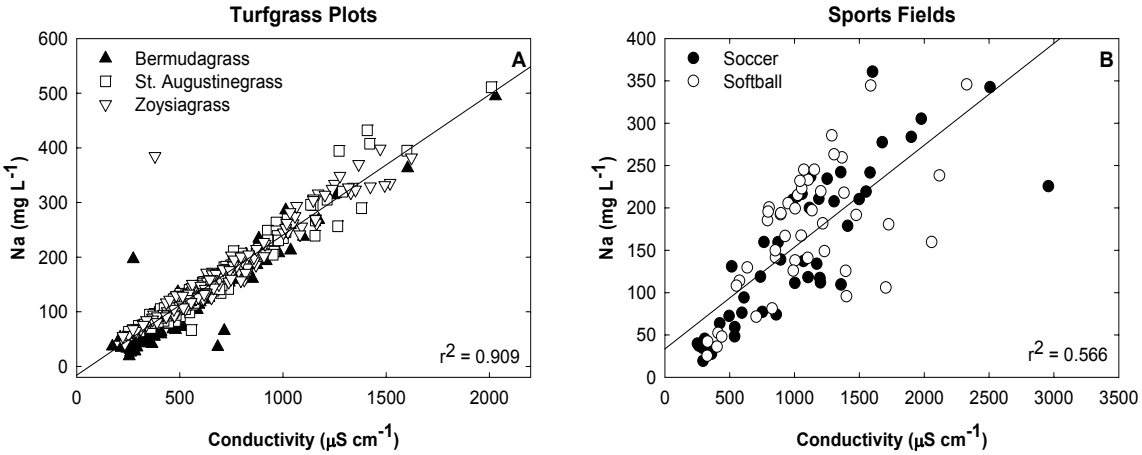

Fig. 15 – The relationship between sodium (Na) and electrical conductivity in soil solution samples from three turfgrass plots (A) and drainage water samples from two sports fields (B).

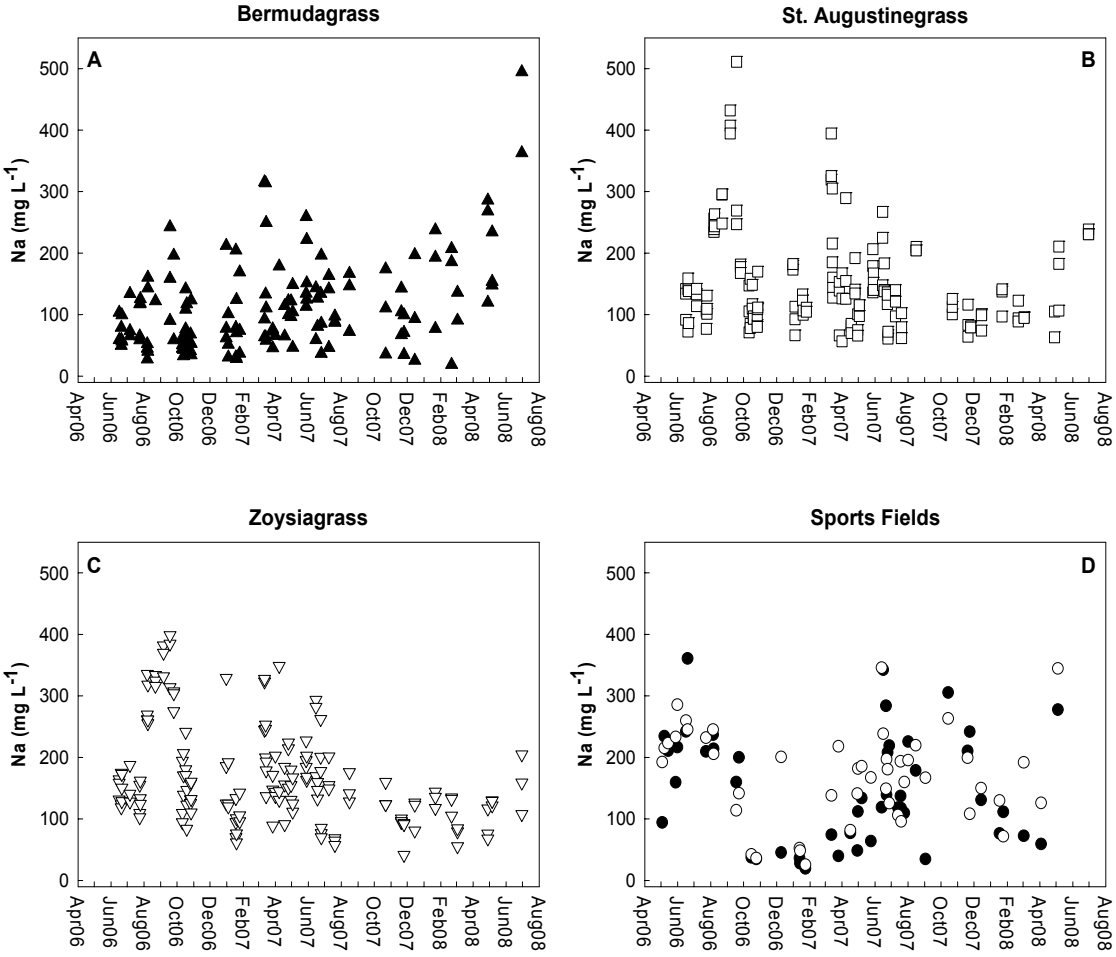

Fig. 16 – Sodium (Na) in soil solutions and drainage water samples. Na concentrations for soil solutions from the Bermudagrass (A), St. Augustinegrass (B), and Zoysiagrass (C) plots and drainage water from the soccer and softball (D) fields. The individual sample values are plotted for each date.

nutrients, during several rainfall events. Rainwater contained low concentrations of nutrients (DOC peak of 8.1mg L^{-1}, DON peak of 1.5mg L^{-1}, and DOP peak of 0.2mg L^{-1}), but did not contribute significant quantities (i.e., one acre-inch of rain containing 8.1mg L^{-1} of DOC would contribute 1.83lbs or 0.83kg of DOC per acre; 1.5mg L^{-1} of DON would contribute 0.34lbs or 0.15kg of DON per acre; and 0.2mg L^{-1} of DOP would contribute approximately 0.05lbs or 0.02kg of DOP per acre). In addition, no deficiencies in concentrations of micronutrients were observed nor were any seasonal patterns ascertained (data not shown).

DISCUSSION

The soil solutions from the Boonville soil typically had much higher concentrations of DOC than the drainage water samples from the sand-based soil. Among the soil solutions, those from the St. Augustinegrass plot appeared to yield the highest amounts of DOC throughout the study, especially during 2007. This may be due

in part to the high thatching potential for St. Augustinegrass that might have occurred because of the concurrent fertilization study (Duble, 1996a). Thatch production can be stimulated by fertilization, and over time, it might be expected that some if not most of the thatch material would enter the soil profile and be subjected to microbial degradation. This added carbon substrate may have led to increased DOC levels. However, mid-way through the study, the St. Augustinegrass began to die off and the turf became increasingly weedy. The weeds were mowed down in early March of 2007 and the St. Augustine grass returned. The weedy material was left on top of the soil and most likely contributed organic carbon to the upper soil profile. This may be another reason for the higher DOC concentrations observed in the St. Augustine plot in 2007.

I also observed that there were lower quantities of DOC in the drainage water samples from the sand-based sports fields. The lower concentrations of DOC in the drainage water samples may be attributed to less organic matter (OM) (Table 1) and perhaps to the lower adsorptive capacity of the sand used to construct the root zones (i.e., less potential for soils to retain DOC). The regular irrigation of the sports fields between rainfall events could have also led to a "flushing" of nutrients through the soil profile, preventing the accumulation of DOC. I had expected to see some seasonal differences between the sports fields and the turf plots because of the over-seeding of the sports fields during the winter months with a cool-season grass. The sports fields were in essence maintained with active vegetation throughout the year and as such, I expected to see higher levels of DOC in the sports fields during the winter months due to the continuous activity of the grass root system. However, the over-seeding did not seem to

contribute much DOC, during the winter months as indicated by the low DOC content of the drainage water samples. This may be due to the fact that while C3 grasses remain green and growing during cool weather, their actual productivity is not that great, since their growing season does not begin until early spring (Duble, 1996b). In fact, the spike in DOC observed during early fall in the sports field may be due to an increase in accumulated biomass from the ending of the C4 grass growing period and the beginning of the C3 grass growing cycle (Jacobo et al., 2000). The potential for increased root activity during the fall in these highly engineered sports fields may also have led to the higher observed DOC concentrations (Xiong et al., 2006). It is clear that the DOC concentrations of the soil solutions and drainage water samples corresponded with the active growth periods of the warm-season turfgrasses. Overall, DOC concentrations found in our study are comparable to results reported by other researchers in agricultural and forest ecosystems (Appendix 3), and do not appear to be extremes when considering the environmental conditions.

I was also able to characterize DON concentrations in the soil solutions from the native soil of the turfgrass plots and drainage water samples from the sand-based root zones. Amounts of DON in both soil solutions and drainage water samples showed seasonal variation similar to that of DOC. The DON concentrations in the soil solutions from the turfgrass plots were much lower than those of the drainage water samples. The turf plots were not continually fertilized or irrigated, and with the exception of the nitrogen fertilization experiment performed on the Zoysia and St. Augustine plots in 2007, no additional fertilizer was applied during the course of this study. The three

turfgrass plots were generally similar with regards to concentrations of DON in the soil solutions, but in some cases the Bermudagrass plot had higher quantities of DON than either the Zoysiagrass or St. Augustinegrass plots. During June and July of 2007, College Station, TX experienced a high amount of rainfall (24.3cm), and on many occasions we observed standing water mainly in the northwest corner of the Bermudagrass plot, but not in the other plots. The greater concentrations of DON observed in the Bermudagrass plot may have been due to the solubilization of DON when the soil became waterlogged and released entrapped organic matter from micropores (Zhang et al., 2004).

The lower adsorption capacity of the sand-based root zones may have contributed to the increased amounts of DON in the drainage water samples, especially shortly after fertilizer applications. However, we observed such high levels of DON in the sand-based soil that the exact source was not readily identifiable. Concentrations of DON in the drainage water samples were similar between the soccer and softball fields, indicating that the differing types of activity did not appear to influence DON. Concentrations of DON in both the soil solution and drainage water samples demonstrated a seasonal rise and fall that appeared to be associated with either the growing seasons of the turfgrasses in the native soil and sand-based root zones, or in the case of the sports fields, also the timing of the fertilizer applications (See Fig. 13). DON concentrations in our study also appear to be comparable to research found in agricultural and grassland ecosystems (Appendix 3).

I was also able to differentiate between the soil solution from the Boonville soil and the drainage water from the sand-based root zones, based on a comparison of the correlations of DOC to DON. The native soil had a much higher correlation ($r^2 = 0.261$, $p < 0.001$) of DOC:DON than the sand-based soils ($r^2 = 0.071$, $p = 0.009$). This may be attributed to the low levels of fertilization applied at the turf plots, as well as the higher levels of OM found in the native soil, leading to a more "stable" soil environment (Sá et al., 2001). When data were analyzed following log transformations, the correlations appeared to be stronger, $r^2 = 0.282$, $p < 0.001$ and $r^2 = 0.174$, $p < 0.001$, respectively, indicating that the high variability due to seasonality of the samples may have been masking the relationship between DOC and DON. The Bermudagrass plot, which was not part of the concurrent nitrogen fertilization experiment, had the strongest correlation between DOC and DON ($r^2 = 0.518$, $p < 0.001$). The St. Augustinegrass plot, which experienced die-off, and over-growth of weeds, and received nitrogen fertilization, had the lowest correlation ($r^2 = 0.207$, $p < 0.001$), which was similar to the soccer field ($r^2 = 0.179$, $p = 0.003$). Overall, the soil solutions from the native soil had a higher DOC:DON ratio (≈16.0:1), which was similar to results reported by Kopp and Guillard, 2004, than the drainage water from the sand-based sports fields. However, this may be attributed to the lower amount of DOC observed in the drainage water samples due to the "flushing" of the soils between rainfall events by irrigation.

Ratios of DOC:DON for the sand-based soils were typical of biological organisms (Wallander et al., 2003; Friedel and Gabel, 2001), albeit rather low (≈5.4:1), but at some points the ratios were as low as 1:1. These low DOC:DON ratios may have

been due to several factors: the difference method used for DON does not account for all forms of nitrogen, and we cannot say with absolute certainty that the calculated DON was specifically DON only; and the addition of fertilizers that were primarily inorganic may have also lead to decreased DOC:DON ratios (Currie et al., 1996), possibly due to assimilation of the inorganic N by the microbial biomass.

There was a strong relationship between DOC and the color of the solutions ($Abs._{440nm}$), which indicates that this may be a fairly simple and inexpensive proxy method for determining levels of DOC in a water sample. However, we cannot positively say what portion of the DOC fraction we were detecting, and we are still unclear as to how effective this method may be in all other ecosystems (i.e., lake water next to a forest, streams, ocean water, etc.). In this study, the color determination appeared to be quite effective for a quick estimation of DOC in native soil solutions (DOC mg L^{-1} = 241.3 x A; where "A" is the absorption reading at 440nm) and sand-based root zone drainage water samples (DOC mg L^{-1} = 371.2 x A).

Dissolved organic carbon (DOC) and BCA/reactive C (BCA-C) were also highly correlated, and this suggests that the bicinchoninic acid assay may be another good proxy indicator of DOC in soil solutions and drainage water samples. However, it is not clear which particular components of the DOC are reacting with the BCA reagent. The BCA assay has been used extensively as an indicator of proteins (Smith et al., 1985), but has also been used for the detection of microbially derived carbohydrates (Joergensen et al., 1996). However, the consistent fraction of DOC detected by BCA (9 to 12% of DOC; Table 3) in our study may indicate that the assay is also reacting with other

materials such as proteins, as indicated by prior studies (Brown et al., 1989). The reagent might also be reacting with humic substances that have bound with amino acids (Keil and Kirchman, 1993), as well as any dissolved substances that can reduce copper. Data from our lab indicated strong reactions between the BCA reagent and tannic, caffeic, and humic acids, but not with coumaric acid (Zuberer, personal communication).

The lower percentage of BCA-C in the DOC (9.8%) from the sand-based root zones might be explained by the relative increase in movement of DOC through the sandy soil. Increased movement of DOC in the sand-based soil, which results from larger pore spaces, higher rates of mass flow, and a lower adsorptive capacity than the native soil, could potentially augment levels of low-molecular soluble carbon (C) in the drainage water samples. Since the sports fields are "flushed" on a regular basis between rainfall events, the DOC may have less of a chance for humification, thereby decreasing the BCA to DOC ratio. Nonetheless, the BCA reagent appeared to detect a relatively constant fraction (9 to 12%) of total DOC, and as such, it may be useful as a quick proxy for DOC in soil solutions or drainage water samples.

Dissolved organic phosphorus (DOP) concentrations in the soil solutions from the native soil were much higher than in the drainage water samples from the sand-based root zones, and showed a marked increase during late summer of 2007 in both systems. During this time period, as mentioned earlier, there were several high rainfall events, and it is possible that the water-saturated soils developed anoxic and reducing conditions, which may have led to greater solubilization of organic phosphorus (Zhang et al., 2004). The rapid rewetting of the soils may have also resulted in the lysis of microbial cells,

releasing otherwise immobile forms of phosphorus (Turner and Haygarth, 2001). The highest concentrations of DOP were typically found in the soil solutions of the Bermudagrass plot, with the exception of the St. Augustinegrass plot in late 2007. The quantities of DOP observed in the Bermudagrass plot may be partially explained by the prior composting study that occurred in 2001. While that particular study was initiated five years earlier than this study, and the wells were placed in control (no compost added) plots, it is possible that nutrients from the incorporated composts have dispersed throughout the plot, especially considering that the Bermudagrass clippings were continuously returned to the soil after the composting study was completed.

The drainage water from sand-based root zones had very low levels of DOP, and on average the softball field had the highest concentrations. It has been shown that phosphorus has the potential to accumulate in soils (Turtola and Yli-Halla, 1999), and with the continuous application of fertilizers to the sports fields, we had thought there might be higher residual levels of DOP. However, the low adsorptive capacity, low levels of OM in the sand-based soils, and the constant irrigation of the fields may have contributed to the low levels of DOP detected. The higher levels of DOP found in the softball field might possibly be explained by the increased amount of bare playing surface, since the infield consists of bare soil. Fertilizer may have inadvertently been applied to the infield, while fertilizing the outfield. The over-sprayed fertilizer on the bare infield would have a "direct" route to the drainage system. The soccer field, however, is completely covered with grass year round, and this increased amount of vegetation could potentially be responsible for a higher uptake of phosphorus. The

relative age of the sports fields may also be a factor, since the soccer field was established five years prior to the softball field, leading to increased levels of OM (as observed when soil core samples were extracted from each field), which could have resulted in the increased incorporation of DOP into the soil. Regardless of the soil or turfgrass type, seasonal variations in DOP concentrations were observed in the soil solutions and drainage water samples, with the highest amounts found during late summer to early fall. Overall, concentrations of DOP found in our study were comparable to results from grassland and forest ecosystems (Appendix 3).

I found some seasonal trends for DIN in the soil solutions from the Boonville soil and in the drainage water from the sand-based sports fields. However, I did notice a marked increase in DIN at all locations during April to August of 2007. The St. Augustinegrass and Zoysiagrass plots showed a marked increase in DIN levels in April, immediately after the implementation of the N fertilization study, which occurred in February and March of 2007. Then in August of 2007, after the high rainfall events, DIN concentrations increased in the soil solution samples from the three turfgrass plots, reinforcing the idea that water-logging the soils can increase the mobilization of nutrients. The sports fields, which were fertilized throughout the year, also showed increases in DIN in April of 2007 (See Fig. 13). This is likely due to the increased fertilizer applications during the spring months as the sports-field managers prepared the fields for increased activity. I also observed high concentrations of DIN for the sports fields during the summer months of both 2006 and 2007, possibly indicating continued fertilization of the fields. In August 2007, there were higher quantities of DIN than in

August of 2006, indicating the influence of the increased rainfall events. However, the increases in DIN did not always coincide with the reported fertilization schedule, and this may have been caused by a failure to report all fertilization events, or possibly an unintentional miscalculation of the amount of fertilizer applied.

Concentrations of DIP were markedly higher in the soil solution samples from the Boonville soil, than in the drainage water from the sand-based root zones. The Bermudagrass plot typically had the highest concentrations of DIP, but it also exhibited the greatest variability. DIP in solutions from the native soil tended to steadily decline throughout the study, but increased in August of 2007. As discussed above, the high amounts of rain may have been a significant factor for this increase. This is probably not the only reason for the steady decline in DIP, but may be one of several environmental factors influencing DIP.

The drainage water from the sand-based root zones had much lower levels of DIP, but did exhibit seasonal variations in quantities of DIP, with the highest levels observed at the softball field in 2006. The relative age of the fields may have been a factor, considering the softball field was established only a year prior to this study. The newly sodded sand-based sports field, with a low adsorptive capacity and low levels of OM may have led to increased recovery rates of DIP from the softball field throughout the study.

In summary, during the late summer to early fall time period, it appears that organic nutrients are at their highest concentrations in soil solutions and drainage water samples from the native soil and sand-based root zones respectively. The

concentrations of DOC, DON, and DOP observed in our study are comparable to other research in varying and similar ecosystems (Appendix 3). A number of environmental factors (i.e., root growth, soil moisture, temperature, aeration, pH, microbial activity, etc.) likely contribute to the observed seasonal variations in organic nutrients. With regards to DOC and DON, seasonal variations were observed at all locations, with the highest levels of DOC found in the soil solutions from the native soil and the highest quantities of DON observed in the drainage water samples from the sand-based soil. DOP demonstrated seasonal variations as well, and the highest observed concentrations were in the soil solutions from the native soil.

CONCLUSIONS

Concentrations of DOC, DON, and DOP were determined in soil solutions under three warm-season turfgrasses grown on a native soil and in drainage water samples from under two sand-based sports fields. Soil solutions and drainage waters have the potential to move off site and into "natural" hydrological ecosystems, which could lead to the impairment of waterways, due to the increased potential for both eutrophication and pathogen survival (i.e., the introduction of nutrients that are available for microbial uptake), and as such, further research is needed. Home owners should be encouraged to have their soils analyzed for quantities of preexisting nitrogen, phosphorus, etc., to understand how much is already there, and how much needs to be added for adequate soil fertility. The high DON concentrations observed at the sports fields, during fertilization events, indicates that sports-field managers also need to be careful with fertilizer applications and mindful of the fact that in addition to fertilizer nutrients, dissolved organic nutrients may also move through the soil profile and into the drainage system. Identifying a mechanism to recycle at least some of the drainage water may alleviate this problem for sports field managers and may have the potential to lower costs over the long run with fewer fertilization applications. However, the potential for accumulation of sodium (Na) and other salts with water reuse may mitigate against this practice, especially where municipal water supplies are high in Na as they are in College Station, TX.

CHARACTERIZATION OF MICROBIAL COMMUNITIES UNDER MANAGED TURFGRASS SYSTEMS

INTRODUCTION

Encroachment of urban areas into rural lands, has led to alterations in ecosystem functions both in the soil and in water. Recent research has demonstrated that soil microbial populations vary in community structure and functionality under disturbed and undisturbed areas of a golf course (Bigelow et al., 2002). In relative terms, this indicates that with different functionality, the potential for microbial processes in the soil profile (i.e., methanogenesis and denitrification) changes depending on the location, which can have both positive and deleterious consequences. Runoff of organic nutrients from urban environments can also potentially influence the microbial populations in waterways by providing a food source for microbes that exist in an otherwise nutrient-poor environment. This may have consequences with regards to pathogen survival and eutrophication (Bretter and Höfle, 1992; Carpenter et al., 1998a). Research is needed to identify the changes that are occurring in microbial populations due to alterations in ecosystem functionality as the urban environment expands. The purpose of this particular study was to characterize the indigenous microbial populations from a native soil and two sand-based root zones; to identify the diversity of microbial populations in the soils under differing types of turfgrasses; and to identify any observable seasonal trends with regards to microbial populations in native soil and sand-based root zones.

MATERIALS AND METHODS

The study took place on the Texas A&M University Campus (West Side), College Station, Texas. Three established turf plots on a native soil, consisting of Bermudagrass (*Cynodon dactylon* (L.) Pers), Zoysiagrass (*Zoysia japonica* Steud.), and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) were sampled. The native soil is a Boonville fine sandy loam (fine, smectitic, thermic Chromic Vertic Albaqualfs) formerly under pasture (Wright et al., 2007). The Bermudagrass plot was established in 2001 as part of a compost application study that ended in 2002. The Zoysiagrass and St. Augustinegrass plots were established in June of 2002, and they were used in a nitrogen fertilization experiment that occurred in 2007, during the present study (Appendices 1a and 1b list fertilization rates applied to each plot). Two sports fields with sand-based root zones, the Texas A&M University soccer and softball fields, were also sampled. The soccer field consists of TifSport Bermudagrass for most of the year and is over-seeded in the winter months with a cool season ryegrass (*Lolium perenne* (L.); 3-way blend). The softball field consists of TifWay 419 Bermudagrass for most of the year and is also over-seeded in the winter months with a cool season ryegrass (*Lolium perenne* (L.); 3-way blend). Both sports field sand-based root zones were established according to the Airfield® system (Airfield Systems, Edmond, Oklahoma), with a geo-textile under-layer to aid in field drainage (Fig. 3a). The soccer field was established in 2000, whereas the softball field was established in 2005. Fertilization of the sports fields occurred at the beginning, middle, and end of each sports season (Appendices 2a and 2b), and irrigation of approximately 5.0cm (2.0in) was

applied to each field every 8 to 10 days unless there was a rainfall event (Craig Potts, Assistant Manager, Grounds Maintanence, Texas A&M University, personal communication).

After mowing, clippings were left on the ground at all locations, except during the fertilization experiment conducted on the Zoysiagrass and St. Augustinegrass plots in early 2007. Mean annual rainfall and temperature in College Station are 101.0cm and 19.8º C respectively, with mean temperatures ranging from a low of 4.3º C to a high of 35.3º C.

Three arbitrarily selected 2.5-cm diameter core samples (15.0cm deep) were extracted from three different locations within each of the turf plots and sports fields every three months for a period of one year (Fig. 17). Core samples were homogenized from each location, giving a total of fifteen composite samples between the five areas. After collection, samples were immediately analyzed for carbon source utilization (CSU) profiles and culturable microbial populations, and the remaining portion was stored at -80ºC for subsequent microbial community analyses by fatty acid methyl ester (FAME) profiling.

Additional soil core samples, 2.5cm in diameter and 15.0cm deep, were collected in October of 2007 at each location for determination of soil organic C and N (Table 1). Populations of bacteria and fungi were enumerated using standard dilution plate count procedures (Zuberer, 1994). Ten grams of each composite sample were placed in sterile 95.0-mL water blanks within two hours of collection. The samples were then shaken by hand until the sample was thoroughly mixed, and allowed to settle for 3-5

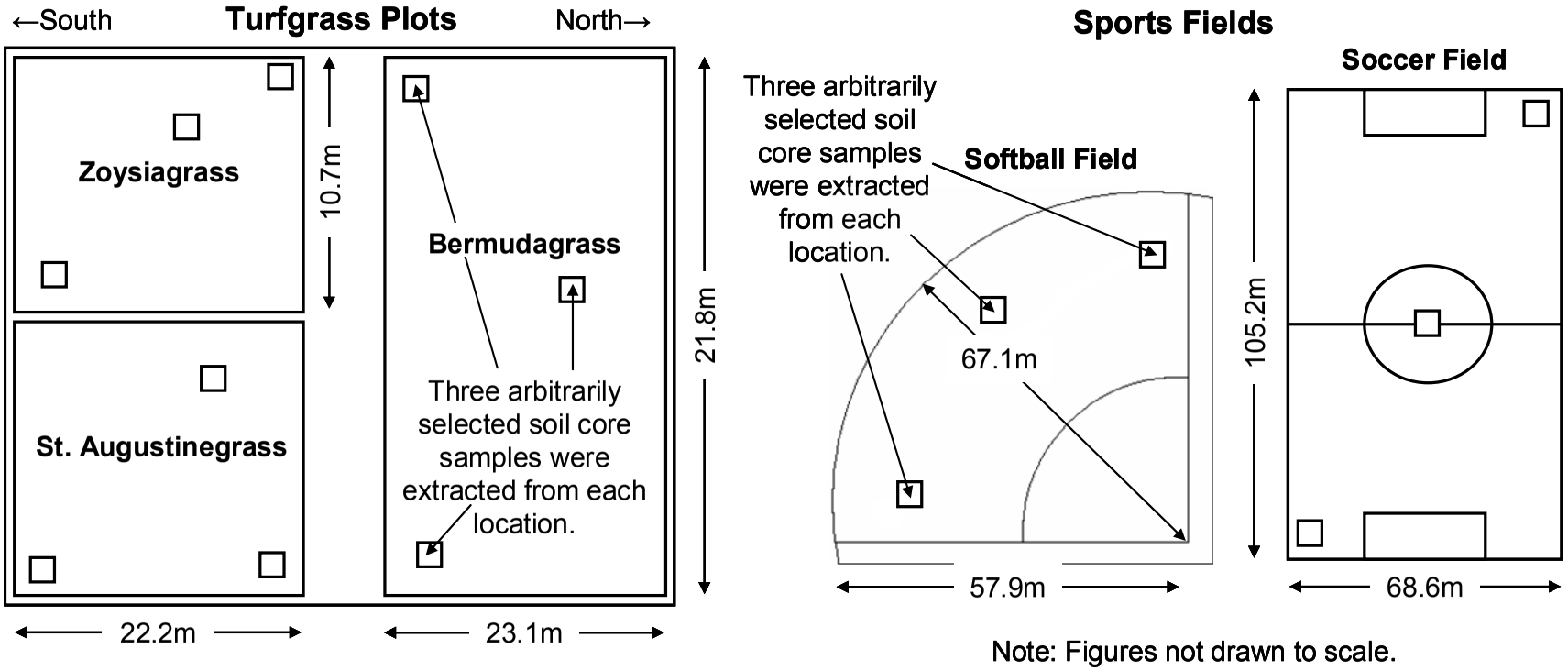

Fig. 17 – Soil core sampling locations. Three core samples were extracted from three areas (each sampling area is indicated by a ☐) at each location. Samples were homogenized, giving a total of fifteen representative samples.

minutes. A ten-fold dilution series was prepared, ranging from 10^{-2} to 10^{-6} using 9.0-mL sterile distilled water blanks.

Aliquots (0.1 mL) were spread plated onto Rose-Bengal Streptomycin agar (Martin's medium) plates (three replicate plates per dilution) to enumerate fungal populations or onto 10% Tryptic Soy Agar (TSA; Difco) plates to enumerate bacterial populations. All dilutions and plating were carried out in a laminar flow hood. Plates were incubated for seven days at room temperature and then analyzed for colony development.

Microbial carbon source utilization (CSU) was analyzed with Biolog® GN2 plates (Biolog Inc., Hayward, California), in an attempt to identify possible differences in microbial community functionality in the native soil and sand-based root zones, as well as to determine any observable seasonal trends in microbial usage of carbon substrates. The 95 carbon substrates found in the Biolog® GN2 plates are listed in Appendix 4. Biolog plates were inoculated with a 10^{-3} dilution by placing 150.0 µL into each of the 96 wells of the GN2 plate using a multi-channel pipetter. The plates were incubated for five days at 32.0º C, and were read using a Biolog plate reader at twenty-four hour intervals, with an initial 0 time. Biolog data were analyzed using average well-color development (AWCD) and standard principal component analysis (PCA) procedures (Garland and Mills, 1991) with PC-ORD 5® software (MJM Software Design, Gleneden Beach, OR). The AWCD was calculated as the mean difference among values of the 95 response (sole carbon source) wells, *R*, and the value of the control well (without a carbon source), *C*. AWCD = $[\sum(R\text{-}C)]/95$. Plate readings for

analysis were chosen based on all plates having an AWCD greater than 0.4. All dilutions and plate inoculations were done in a laminar flow hood, within three hours of sample collection.

A standard fatty acid methyl ester (FAME) method (Cavigelli et al., 1995) was used with the stored (-80º C) composite samples to identify differences in microbial community structure between the various grasses and soils, as well as seasonal changes in community composition. Prior to the beginning of the analysis, all glassware was oven heated, over-night at 100º C to remove any residual fatty acids. Samples (10.0g) of each soil were air dried overnight, and after removal of any plant materials and roots, 3.0g of each sample were placed into glass centrifuge tubes. To lyse the cells in each tube, 15.0mL of a potassium hydroxide/methanol solution (1.09% KOH [w/v]) were added to each sample. Samples were briefly vortexed every 10min, and placed in a 37º C water bath between mixings. After one hour, 3.0mL of glacial acetic acid were added to each tube, and then shaken. Litmus paper was used to verify that the acetic acid had neutralized the solution of each sample before proceeding. Ten milliliters of hexane were then added to each tube to extract fatty acids and shaken by hand. Tubes were centrifuged for twenty minutes at 1,000 x g at 4º C. The supernatant was then removed (approximately 2/3 from each sample) and placed into 9.0mL tubes. The extracts were evaporated under a stream of nitrogen gas until approximately 0.5mL of supernatant remained, which was then transferred to small crimp-top serum vials. Nitrogen gas was continuously applied until all the solvent was evaporated from the vials, and they were then immediately sealed with a Teflon-lined cap and aluminum sealing band. Extracted

samples were sent to the University of Delaware Plant and Soil Sciences Department for analysis using an Agilent model 6890 gas chromatograph with flame ionization detector (Agilent, Wilmington, DE). Two microliters of each sample were injected into a Hewlett Packard (Agilent) Ultra 2 (Crosslinked 5% Phenyl methyl silicone) column 25.0m x 0.2mm x 0.33μm with a 100:1 split ratio and flow rate of 0.6mL/min using hydrogen as the carrier gas. The injection temperature was 250°C, and the detection temperature was 300°C. The initial oven temperature was 170°C (hold for 0.0min) and ramped at 5°C/min to a final temperature of 300°C, for a total run time of 12.0min. Peaks were named using the Sherlock Eukary program (MIDI, Inc., Newark, DE).

Microbial enumeration, CSU profiles, and FAME profiles were all analyzed with SigmaStat 3.5 (Systat Software, San Jose, California), using one-way ANOVA (Tukey's Test and Holm-Sidak Test) to identify any significant differences between data sets. The Shannon-Weaver Index was used as an indicator of diversity for the CSU and FAME profiles. The Shannon-Weaver Index was calculated with PC-ORD 5® as: (H') = $- \sum (n!/N) \times \ln (n!/N)$, where n = concentration of each carbon substrate (well color development) or fatty acid and N = sum of the concentration of all bands of the sample (Marschner, 2007b).

RESULTS AND DISCUSSION

Bacterial and fungal populations demonstrated seasonal trends regardless of soil or turf type. Greatest numbers were observed in early fall for all locations (Fig. 18). The Bermudagrass plot tended to contain the highest bacterial numbers over time among the three turfgrasses on the native soil, and was significantly different at three of the four sampling dates ($p = 0.015$, December 2006; $p = 0.007$, March 2007; and $p = 0.004$, June 2007). The higher bacterial numbers found at certain sampling dates within the Bermudagrass plot, may be due in part to the fact that this particular plot was established a year earlier than the other plots and may have been influenced by the composting experiment conducted prior to this study. While the composting experiment occurred four years prior to our own work, the added compost may have led to the development of a more robust bacterial population (Saison et al., 2006). The more robust population could possibly be due to the surface transport of organic carbon from composted plots to the non-composted control plots sampled in this study. Fungal populations were similar among the three turfgrasses, with the exception of the March 2007 sampling of the Zoysiagrass plot ($p = 0.036$). The softball field had higher counts of both bacteria ($p = 0.044$, March 2007) and fungi ($p = 0.025$, March 2007 and $p = 0.056$, September 2007) than the soccer field. Comparison of the bacterial and fungal numbers between the Boonville soil and the sand-based root zones indicated there were no significant differences in microbial numbers between the two soil types. The lack of significant differentiation between the native soil and sand-based root zones could possibly be due

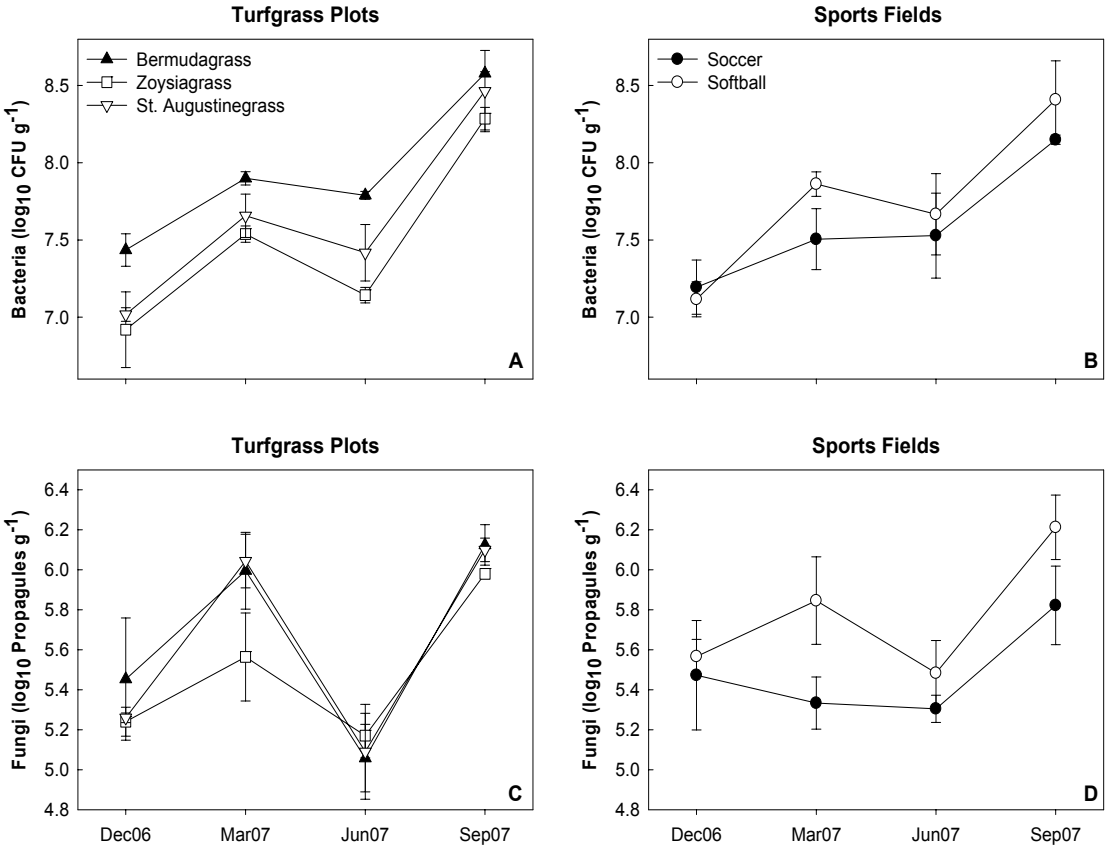

Fig. 18 – Numbers of culturable bacteria and fungi in the native soil and two sand-based root zones. Bacterial counts (colony forming units – CFU) for the turfgrass plots (A) and sports fields (B). Fungal counts (propagules) for the turfgrass plots (C) and sports fields (D). Error bars represent the standard deviation of three replicate samples. Legends are representative of all graphs.

to the inherent problems (i.e., no accounting for anaerobic microbes) associated with culture-based enumeration (Amann et al., 1995). However, it is also possible that the physico-chemical soil conditions were suitable for microbial growth in both environments and may be the reason for similar microbial populations (Griffiths et al., 2008). Bacterial counts in September were significantly greater than all other sampling dates, and March counts were significantly higher than the December sampling date (Tukey's test, $p < 0.05$). Fungal counts for September were significantly higher than the June and December sampling dates, while the March counts were only significantly greater than June (Tukey's test, $p < 0.05$). The augmented microbial populations may be due to the increased root density observed in warm season grasses during early fall (Xiong et al., 2006). The similarities among the turfgrass plots and sports fields may indicate that the Boonville soil and sand-based root zones had comparable physico-chemical soil characteristics. There also appeared to be a seasonal influence on both bacterial and fungal populations.

In addition, this study also indicated that as bacterial populations increased, there was a concomitant increase in fungal populations (Fig. 19). Since the Bermudagrass had a higher bacterial population, I expected to find a relatively larger fungal population in the Bermudagrass plot. This was not necessarily the case, and the concurrent fertilization experiment that occurred during the beginning of 2007 in the Zoysiagrass and St. Augustinegrass plots may have been the reason for the lack of separation between the three turf plots. In summary, although there was an apparent trend toward larger populations in the Bermudagrass plot, overall microbial numbers were not

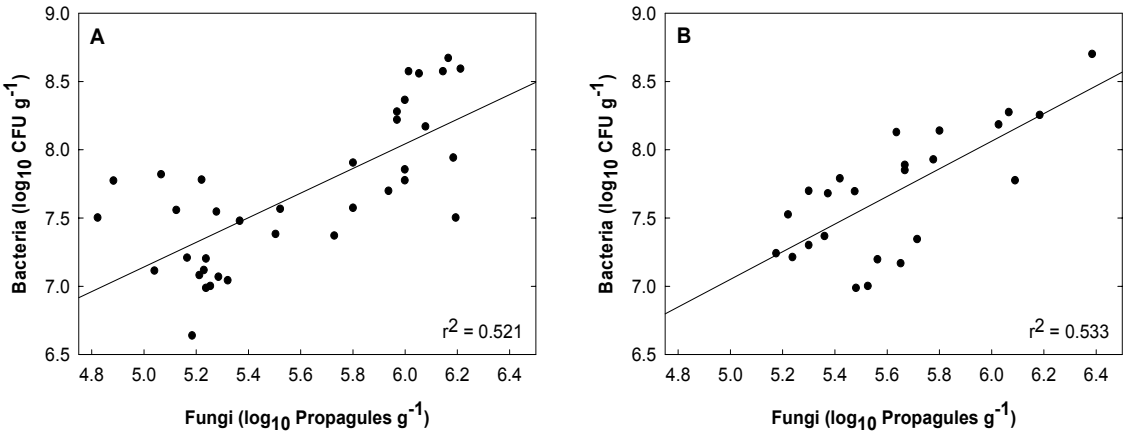

Fig. 19 – Relationship between bacterial and fungal populations in a native soil under three turfgrasses (A) and in sand-based root zones under two sports fields (B). Bacterial colony forming units (CFU) versus fungal propagules.

significantly different among the three turfgrass plots.

The microbial numbers observed for the two sports fields were not significantly different from those of the turfgrass plots. However, when the softball field was compared to the soccer field, the softball bacterial and fungal counts for March 2007 separated from the soccer field counts (Fig. 18). I had expected to see greater differences between the two fields, since the soccer field was established five years prior to the softball field, and had a much thicker layer of organic matter (≈4.0cm found in the soccer field versus ≈2.0cm found in the softball field). I had also expected to find greater numbers of bacteria and fungi in the native soil than in the sand-based root zones. There was no significant difference, however, and in some instances, the sports fields had higher numbers of bacteria and fungi than the turfgrass plots. The slightly

larger populations in the sand-based root zones may have been due to the different extraction efficiencies of the two soils. The native soil contains somewhat higher levels of organic matter (OM) and clay than the sports fields (Table 1), and that typically leads to greater soil aggregate formation, as well as to an increase in adsorption potential. With greater soil aggregation and adsorption potential, microbes can potentially become "trapped" in micropores or adsorbed to fine clay particles, and may not be easily extracted. This could have led to lower extraction of cells from the native soil, and therefore lower observed counts among the turfgrass plots.

Next, I compared all the samples together to look for any differences between sampling dates (i.e., seasonal influences). Significant changes in microbial abundance were observed with regards to the season. The September bacterial counts were significantly higher than all the other sampling dates. During June – July of 2007, College Station, TX experienced an inordinate amount of rainfall. The increased rainfall, in combination with warm temperatures and active grass growth (increased root densities), may have led to a marked increase in bacterial numbers. While we do not have any specific data regarding the rooting densities of our sites, Xiong et al. (2006) have reported that root densities were greatest in September (with regards to their study in Stillwater, OK) for both a USGA designed putting green and an Airfield® system. The March sampling date, which occurred at the beginning of the growing season, showed significantly higher bacterial counts than the December sampling date. This was probably due to the introduction of new carbon (i.e., root exudates, etc.) to the soil from growth of the turfgrasses. In the case of the Zoysiagrass and St. Augustinegrass

plots, the concurrent fertilization experiment, which provided additional nitrogen, may have also led to increased bacterial populations. Fungal populations for the September sampling were also significantly higher than all the other sampling dates, other than March. Populations from the March sampling date were also significantly higher than those in June, but not December. In fact, the June samples appeared to have the lowest fungal counts of any of the sampling dates. Fungal counts have been found to be lower in the early part of the year, and peak in early autumn (Wallander et al., 2001), which is contrary to the peak we observed in March. However, the differences between Wallander et al. (2001) and our own study may be due to the physico-chemical differences in soils found in a forest ecosystem and turfgrass environment. The high counts observed in March may have been due to the warm temperatures experienced in College Station during the spring months in 2007. The low fungal counts in June may once again be due to the high rainfall events that occurred during June – July of 2007. Water logged soils may have led to the development of slightly anoxic or even anaerobic environments with a subsequent decline in fungal populations, since most soil fungi are aerobes (Sylvia et al., 1997; Madigan et al., 2003). Overall, bacterial and fungal populations were comparable among the individual turfgrasses and between the native soil and sand-based root zones, but microbial populations varied significantly across seasons.

There were no apparent differences between turfgrasses or soil types with regards to carbon substrate utilization (CSU) profiles (Fig. 20). The first two principal components accounted for 39.1% of all the data variability, which has been found to be

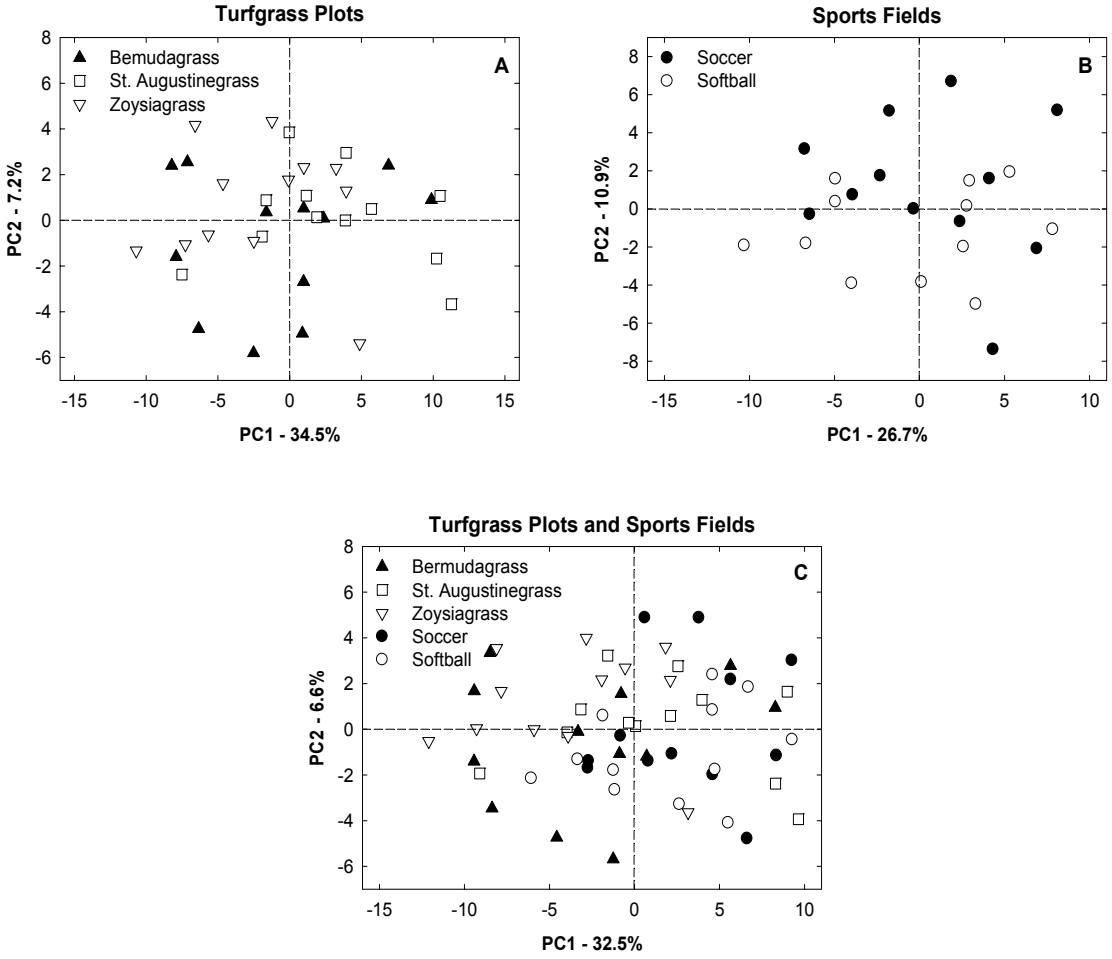

Fig. 20 – Principle component analysis (PCA) of carbon substrate utilization (CSU) profile data for three turfgrass plots (A), two sports fields (B), and combined turfgrass plots and sports fields data (C). The percent variance explained by each principle component (PC1 or PC2) is listed in each axis title.

an adequate amount of variation for comparison of samples (Palojärvi et al., 1997), and no distinct clustering was observed when profiles were compared across all locations (Fig. 20c). However, when the sports fields were analyzed separately (Fig. 20b), there did appear to be some separation between the older soccer field and younger softball field. The lack of clustering observed when looking at the "overall" picture suggests a high degree of similarity in CSU by all microbial populations. Thus, regardless of the location in this study, there appeared to be a high degree of "functional redundancy." Both the turf plots and sports fields had widely distributed points. The data suggest that the microbial communities in these varied systems utilize a similar range of carbon substrates regardless of the soil or turfgrass type, thus the functional diversity of the communities appears quite similar.

However, when the CSU profiles are considered with respect to each individual sampling location (Fig. 21), there appeared to be seasonal variations in all CSU profiles, except for the Bermudagrass plot. The similarity of the Bermudagrass sampling dates may be explained by the composting study that was performed on the plot, as well as the relative age of the plot compared to the other locations. Among the turfgrass plots and sports fields, the September 2007 samples predominantly separated from all other sampling dates, which I thought might be explained as an inoculum density difference. Since I used a 10^{-3} dilution for all samples, there is the possibility that I inoculated the Biolog® plates with a higher density of cells with regards to the September soil samples, which had significantly higher bacterial and fungal counts than any other sampling date (Fig. 18). However, since I used an AWCD to compare all samples, inoculum density

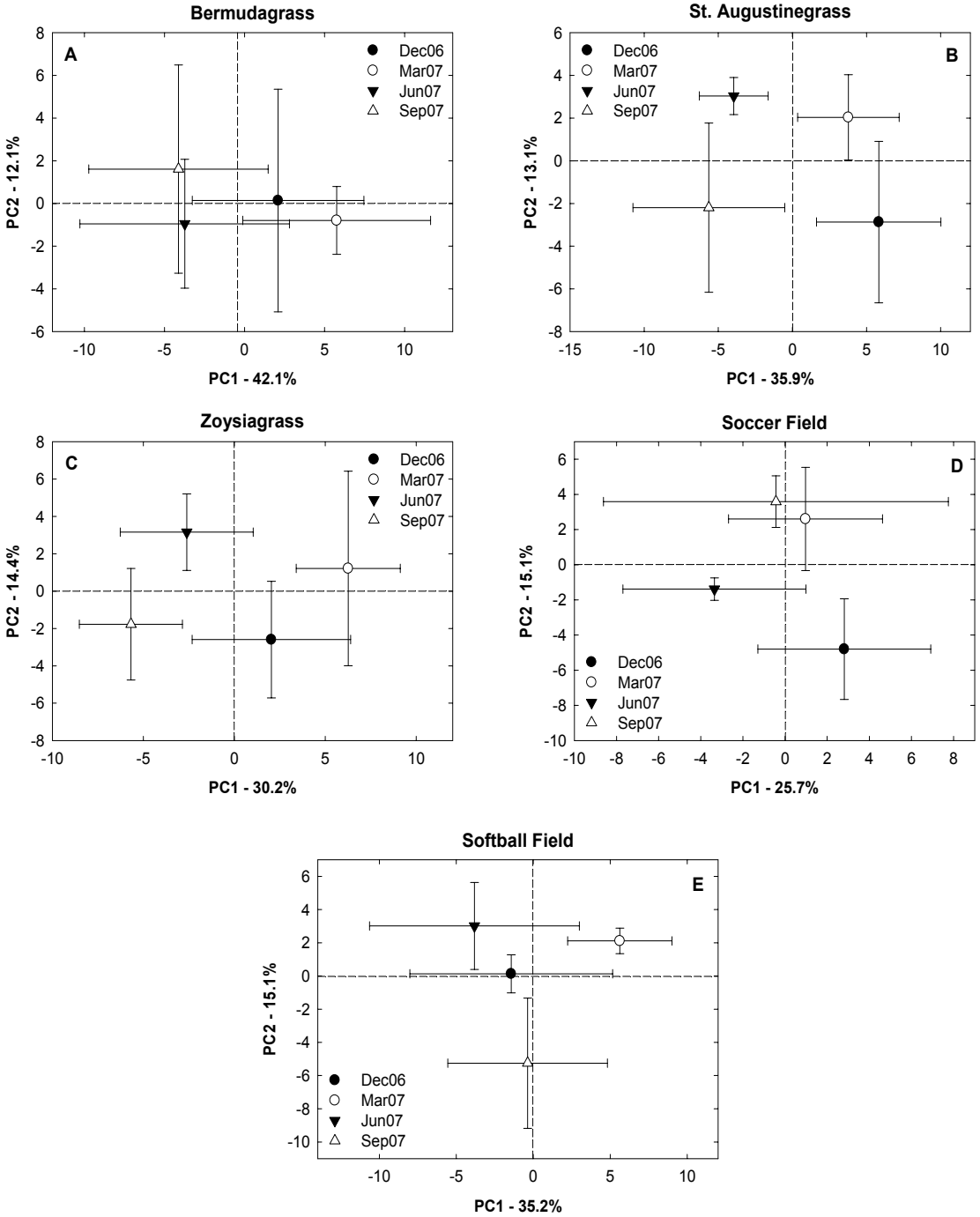

Fig. 21 – Principal component analysis (PCA) of carbon substrate utilization (CSU) profiles over time for three turfgrass plots and two sports fields: Bermudagrass (A), St. Augustinegrass (B), Zoysiagrass (C), Soccer Field (D) and Softball Field (E). The percent variance explained by each principle component (PC1 or PC2) is listed in each axis title. Error bars represent the standard deviation of three replicate samples.

does not seem to be a factor, and the difference in CSU profiles may instead be due to seasonality.

Comparisons of individual sampling locations suggested that CSU profiles varied somewhat with the season. Among the turfgrass plots (Fig. 21), the St. Augustinegrass and Zoysiagrass appeared to have the greatest separation among replicate samples. The differences between sampling dates may have resulted from the fertilization experiment that was implemented at the time of sampling, since it has been shown that CSU can be relatively sensitive to short-term fertilization events (Fließbach and Mäder, 1997). The St. Augustinegrass and Zoysiagrass plots were separated into sampling blocks with different rates of nitrogen fertilization (Appendix 1b), and each of the extracted soil samples was collected from non-control blocks. CSU profiles appeared to be influenced by seasonality among the younger sites, but at the older Bermudagrass plot, it appeared that the microbial communities utilized similar ranges of substrates regardless of the season.

As for the sports fields (Fig. 21), the older soccer field demonstrated some overlap between the March and September 2007 sampling dates, with separation occurring among all other sampling dates. The softball field samples, however, appeared to separate out at each date. The separation observed among the samples collected from both sports fields suggests seasonal variations in community function with respect to CSU profiles. The similarity of the March and September samples from the soccer field, which is covered with Bermudagrass for most of the year, may be explained by the age of the field. As indicated by the oldest site (Bermudagrass plot),

the relative age of the site may influence the overall "uniqueness" of CSU profiles, and could indicate why there was greater separation among the softball field samples than the soccer field samples. The differing types of activity on each of the fields could also potentially influence the CSU profiles. The activity and management on each of the fields is somewhat different throughout the year and may affect CSU profiles, since activities on the soccer field extend well beyond events on the softball field. This could also explain the similarity between the March and September 2007 sampling dates. However, if this were the case, I would also expect to see similarities with the June 2007 sampling date. The CSU profiles did not indicate this and as such, wide scale activity (i.e., soccer cleats aerate and incorporate grass into the soil and increased fertilization and irrigation to keep the field green during the hot summer months) appears to be a non-essential factor for CSU determination. Based on the separation of sampling dates at most locations, it appears that the microbial CSU profiles associated with the turfgrasses grown on the native soil and the grasses on sand-based root zones, are influenced by both seasonality and the relative age of each site. Overall, the similarities in CSU profiles indicate that the microbial communities associated with the turfgrasses grown on the native soil and the grasses on the sand-based root zones, were quite similar with respect to metabolic function.

Whole-soil fatty acid methyl ester (FAME) analysis identified differences in the composition of the microbial communities from native and sand-based soils. FAME profiles differed between the individual turfgrasses, as well as between each of the sports fields (Fig. 22). A listing of the fatty acid markers included in the analysis is given in

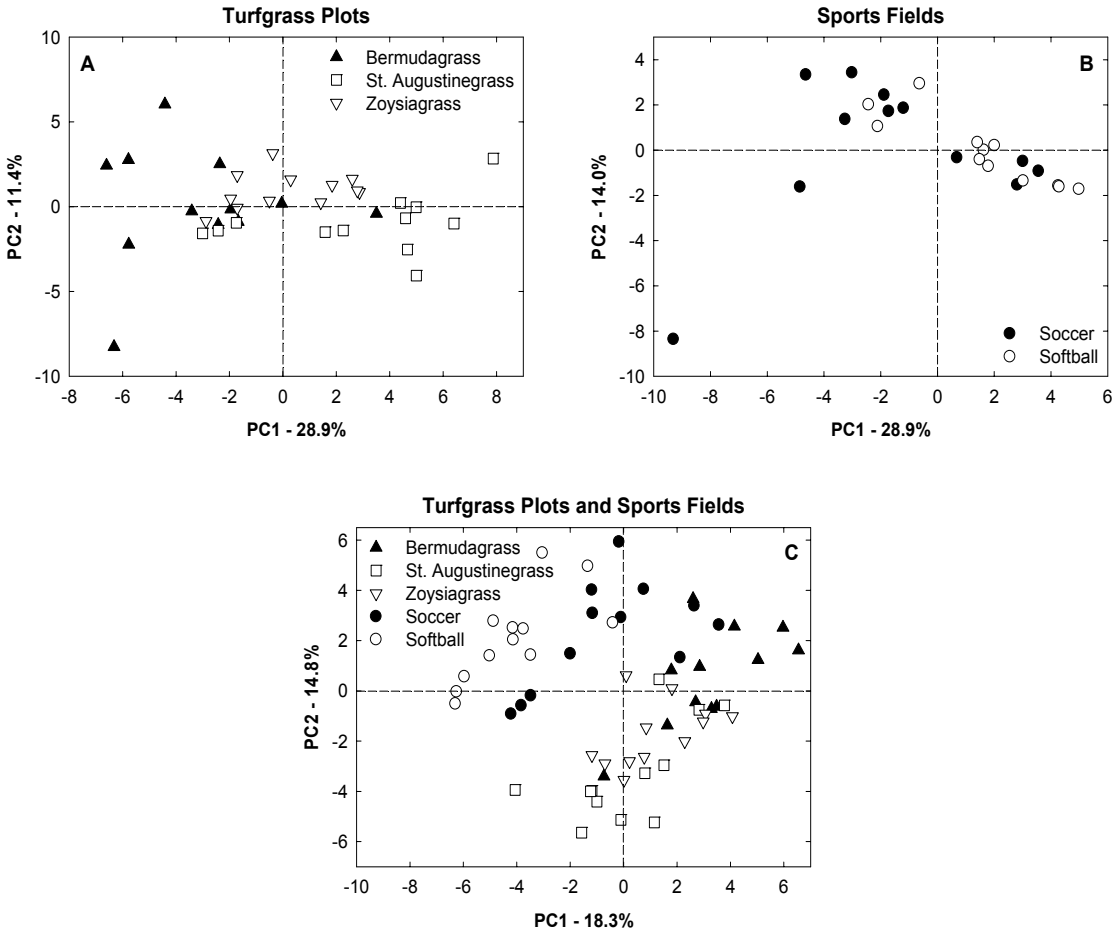

Fig. 22 – Principal component analysis (PCA) of whole-soil fatty acid methyl ester (FAME) profiles of microbial communities from all sampling dates for three turfgrass plots (A), two sports fields (B), and combined turfgrass plots and sports fields data (C). The percent variance explained by each principle component (PC1 or PC2) is listed in each axis title.

Appendix 5. The Zoysiagrass communities appeared to have the greatest similarity among the three different turf plots (points are clustered near the central axes). The FAME profiles from the softball field samples had the greatest similarity within the sports fields, and this may be due in part to the age of the field, when compared to the soccer field.

Ordination (PCA) of the sports-field FAME profiles also indicated differences in the communities of the two fields, with the sand-based root zones clustering separately from the turfgrass plots and from each other (Fig. 22c). The softball field, which was established approximately five years after the soccer field, was clearly separated from any of the turfgrasses on native soil and from the soccer field. Research has shown changes among microbial communities based on the relative age of reclaimed surface-mined lands vegetated with coastal Bermudagrass (Peach, 2001). It is possible that the relative ages of the sand-based root zones may be a contributing factor in the observed differences in FAME profiles between the two sports fields. All of the softball samples tended to cluster in one quadrant, and as in the case of the Zoysiagrass plot, this indicates a similarity in the FAME profiles of the three replicate samples. In addition, the soccer-field FAME profiles clustered separately from the softball field profiles, and with the exception of a few outliers, separated from the turfgrass plots. In fact, the soccer field outliers tended to overlap with data points from the Bermudagrass plot (same species), and this may indicate a shift in community structure among the sports fields, over time. Alternatively, this might also suggest that over time, similarities in microbial communities may develop, and that might explain why the FAME profiles of

the turf-type Bermudagrass (soccer field) and the common Bermudagrass (turfgrass plot) overlap. Evaluation of all FAME profiles identified differences in the composition of microbial communities among the three turfgrasses grown on the native soil. Further, there were also detectable differences in the communities of the two sports fields, despite the similarity of their construction and vegetative covers. It is apparent that community structure can be influenced by age, soil type, and/or turfgrass type, which is comparable to other research (Elliot et al., 2004; Bartlett et al., 2007).

Comparison of the whole-soil FAME profiles at individual sampling dates, revealed differences over time for both the individual turfgrasses and sports fields (Fig. 23). There appeared to be changes in community structure over time, since the sampling dates, especially September 2007, tend to separate out. At each location, the September 2007 sampling appears to be unique. The Bermudagrass plot samples, as in the case of the CSU profiles, tended to separate less than the other turfgrass plots. The similarity among FAME profiles may be due to several factors: the composting study initially performed on the Bermudagrass plot, which may have led to the development of a unique population (Steger et al., 2003; Saison et al., 2006); the age of the Bermudagrass relative to the other plots, since it has been demonstrated that over time microbial populations develop into distinct communities (Peach, 2001); and the physico-chemical properties of the soil underneath the older, established Bermudagrass. The separation of FAME profile sampling dates among the St. Augustinegrass and Zoysiagrass plots may be explained by the fertilization experiment that was performed on these turfgrass plots in 2007, which provided readily available forms of nitrogen that

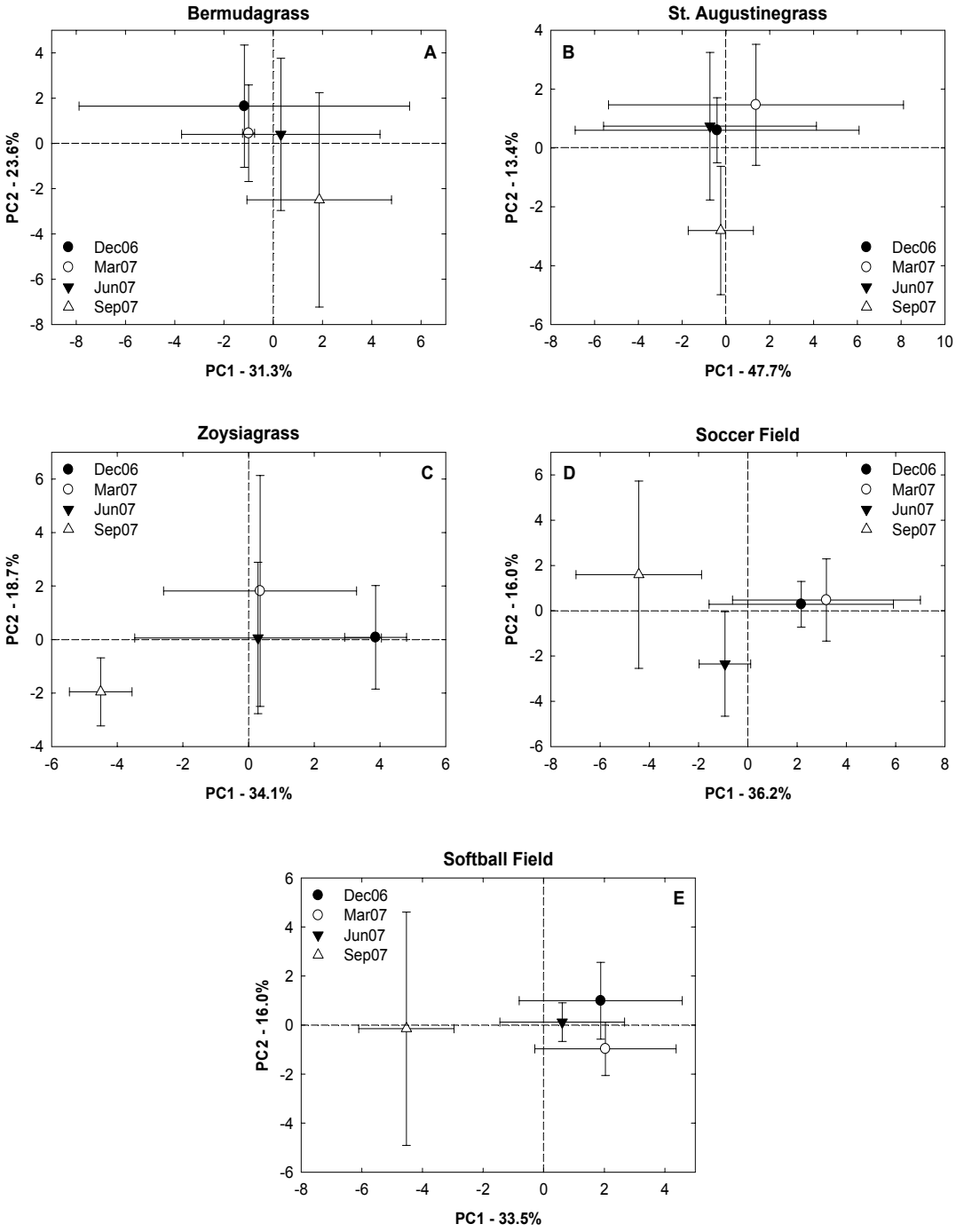

Fig. 23 – Principal component analysis (PCA) of whole-soil fatty acid methyl ester (FAME) profiles over time for three turfgrass plots and two sports fields. Bermudagrass (A), St. Augustinegrass (B), Zoysiagrass (C), Soccer Field (D) and Softball Field (E). The percent variance explained by each principle component (PC1 or PC2) is listed in each axis title. Error bars represent the standard deviation of three replicate samples.

may have influenced the native microbial populations. However, it has also been shown that fertilization may not play as important a role in determining community structure as seasonality (Acosta-Martínez et al., 1999), and as such, the separation between sampling dates may largely be due to seasonality. Therefore, the primary influence on our observed results may be a combination of both seasonality and the physico-chemical properties of the native soil. Analysis of FAME profiles for each location was useful in detecting differences in the composition of microbial communities between the individual turfgrasses over time.

The sand-based root zones of the sports fields also demonstrated differences in microbial community structure at each sampling date, with September 2007 separating from all the other sampling dates (Fig. 23). The soccer field had overlap between all sampling dates, when compared to the softball field, which is similar to the increased overlap observed in the older, established Bermudagrass plot. Possible reasons for the observed differences between the two fields may include: the age of the sports fields could influence the microbial community composition (Peach, 2001); the activity performed on each of the fields could to a lesser extent affect the community (soccer cleats could aerate the soil and lead to increased incorporation of plant materials); and the physico-chemical properties of the older soccer field (greater amount of organic matter – Table 1) might also alter community composition. Overall, observation of the FAME profiles at each sampling date demonstrated unique communities among each of the individual turfgrasses and sports fields at each sampling date, and there did appear to be a seasonal shift in community structure.

Analysis of specific fatty-acid markers (Kaye et al., 2005), as indicators of particular microbial groups (i.e., gram-negative bacteria, gram-positive bacteria, fungi, etc.), at each sampling date revealed similar relative abundances when comparing just the select fatty acids throughout the year (Fig. 24a and b). Regardless of the turf or soil type, bacterial populations appeared to be similar throughout the year, while fungal populations appeared to demonstrate some slight differences between the native soil and sand-based root zones. The unique physico-chemical properties of the two types of soil, may have led to the observed differences in fungal populations.

Analysis of the FAME profiles for these specific fatty acid markers pooled across the seasons revealed very clear separation of the microbial communities associated with the individual turfgrasses and the sports fields (Fig. 25). The specific fatty acid markers identified different clusters for each location. The greatest differences appear to be between the native soil and the sand-based root zones, and this separation may be due to once again, the physico-chemical differences between the native soil and sand-based root zones.

I also attempted to identify differences between the native soil and sand-based root zones, as well as the individual turfgrass and sports fields, based on the diversity of carbon substrates utilized (Appendix 4) and the identified fatty acids (Appendix 5), using the Shannon-Weaver diversity indexes for CSU and FAME profiles (Fig. 26). With regards to CSU, there was a significantly higher degree of diversity in June 2007, than during the March or December sampling dates (Holm-Sidak, $p = 0.006$ and $p = 0.008$ respectively), as evidenced by a larger range of C source utilization. However, there

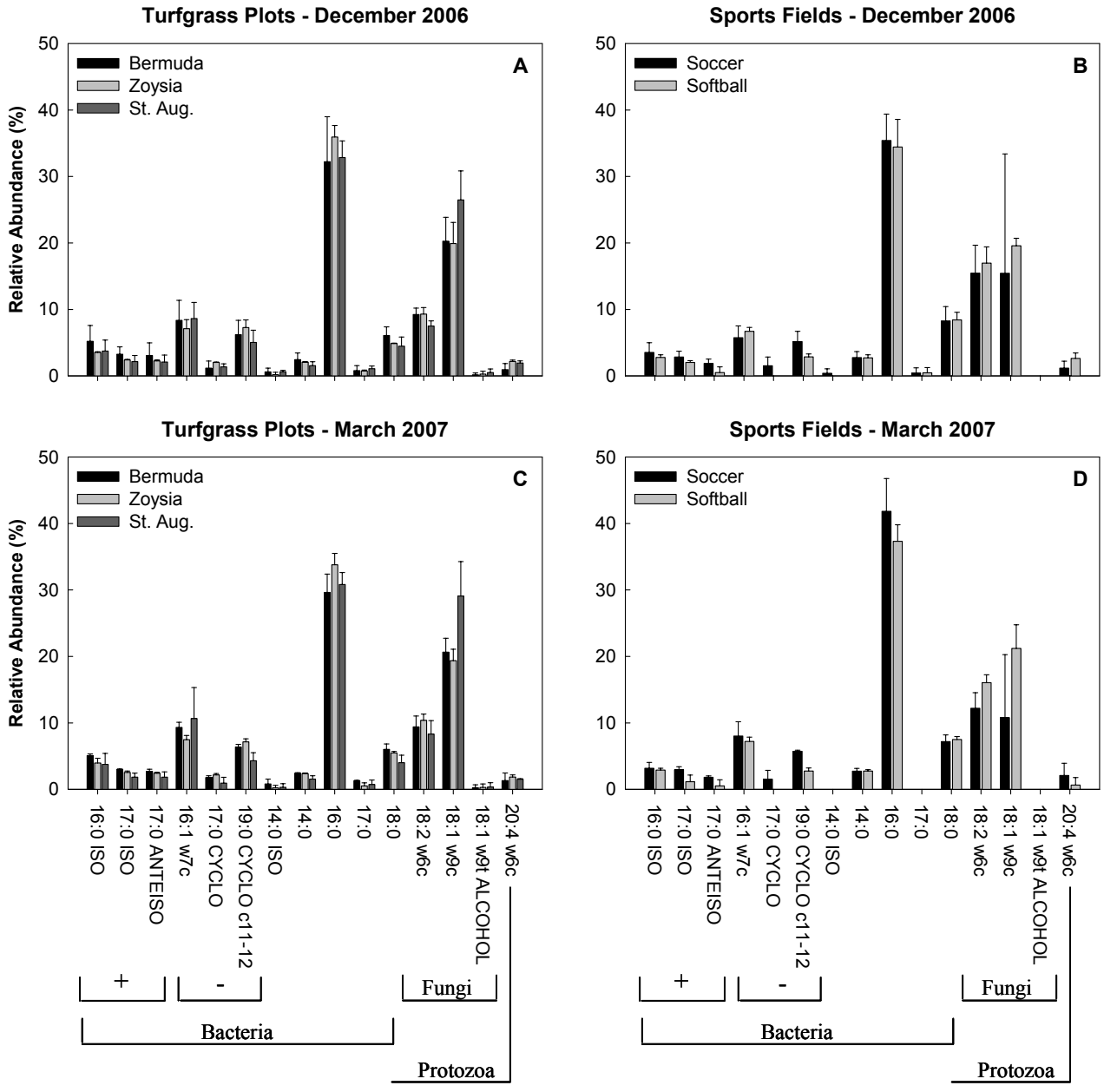

Fig. 24a - Relative abundance of selected marker fatty acid methyl esters (FAMEs) for Gram positive (+), Gram negative (-), bacteria, fungi, and protozoa at each sampling date and location: December 2006 – Turf Plots (A) and Sports Fields (B) and for March 2007 – Turf Plots (C) and Sports Fields (D). Error bars represent the standard deviation of three replicate samples.

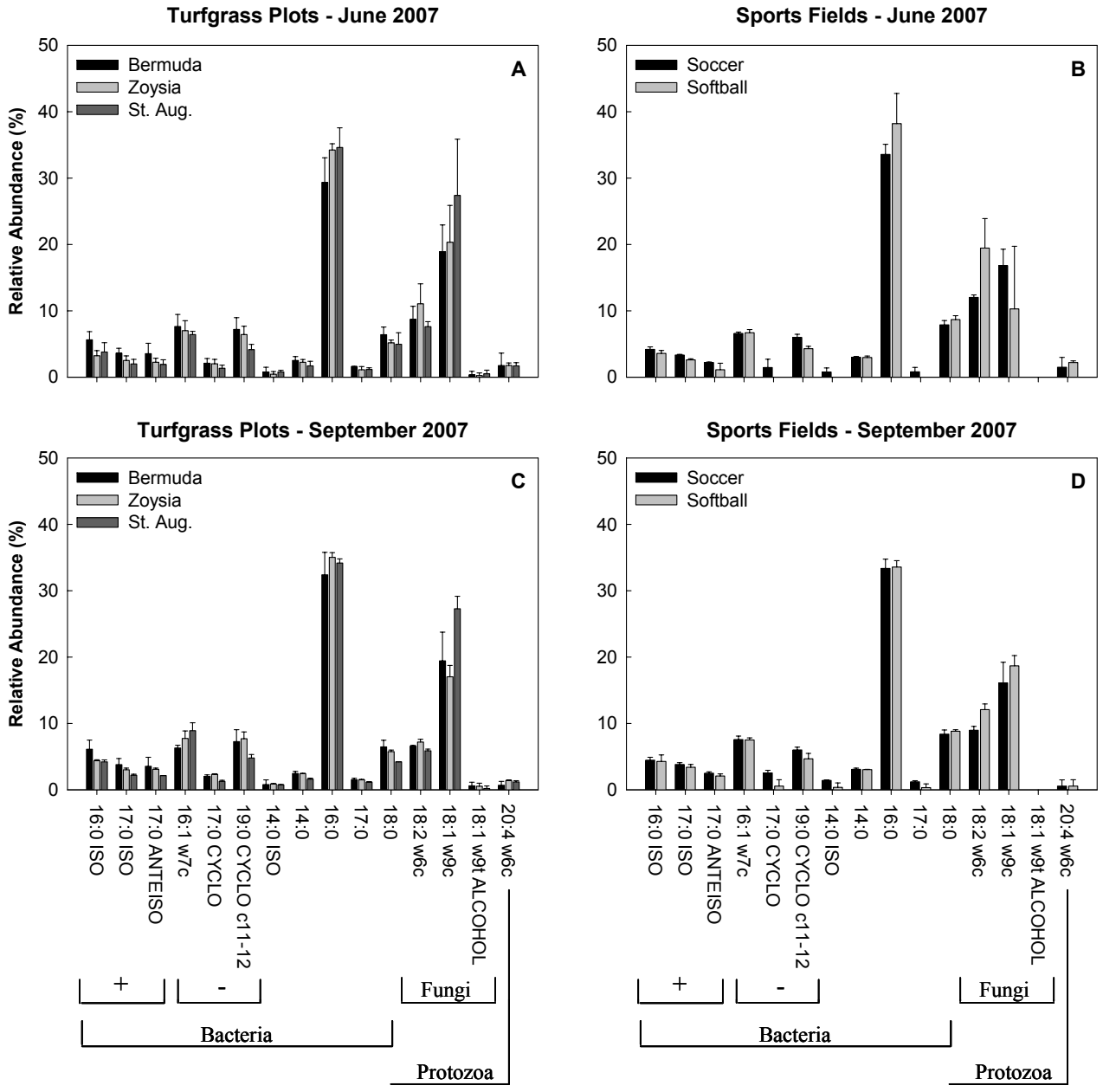

Fig. 24b - Relative abundance of selected marker fatty acid methyl esters (FAMEs) for Gram positive (+), Gram negative (-), bacteria, fungi, and protozoa at each sampling date and location: June 2007 – Turf Plots (A) and Sport Fields (B) and for September 2007 – Turf Plots (C) and Sports Fields (D). Error bars represent the standard deviation of three replicate samples.

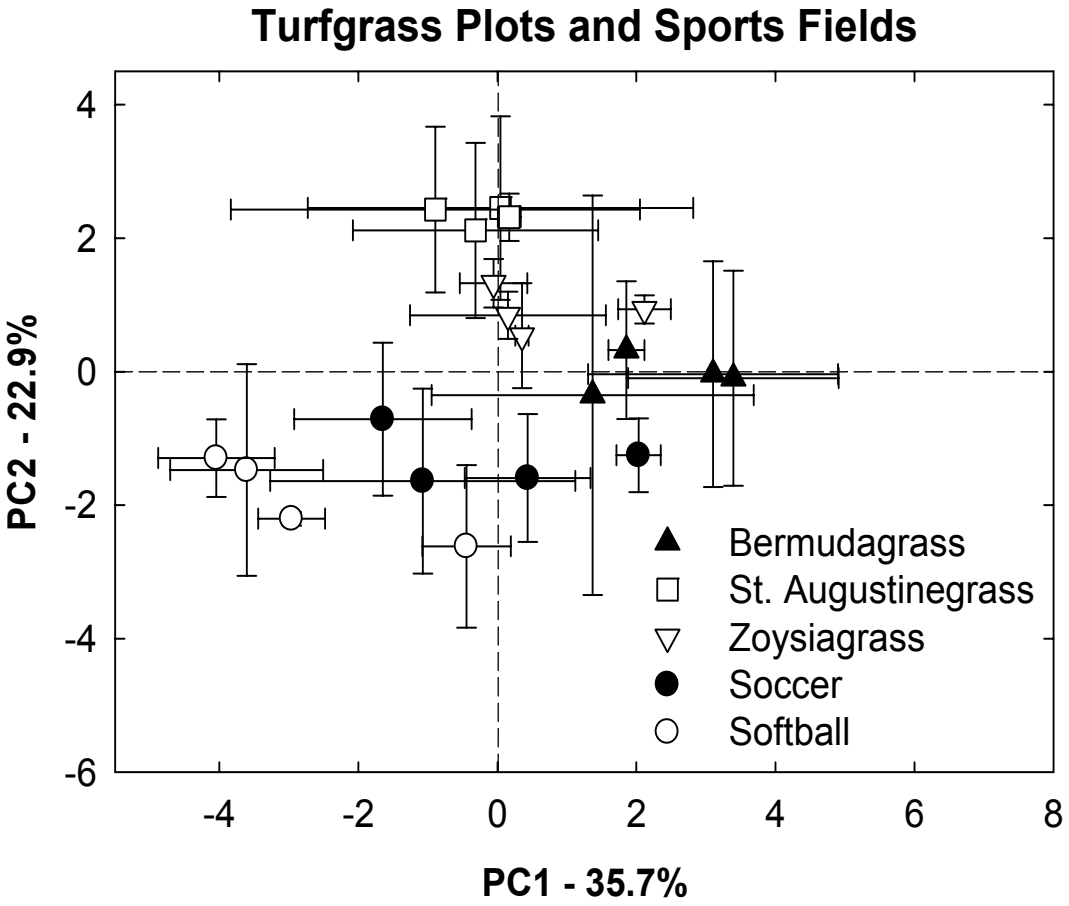

Fig. 25 – Principal component analysis (PCA) of whole-soil fatty acid methyl ester (FAME) data from all sampling dates with selected marker fatty acids. Points represent the mean value of three samples collected from each location at each sampling date. The percent variance explained by each principle component (PC1 or PC2) is shown in each axis label.

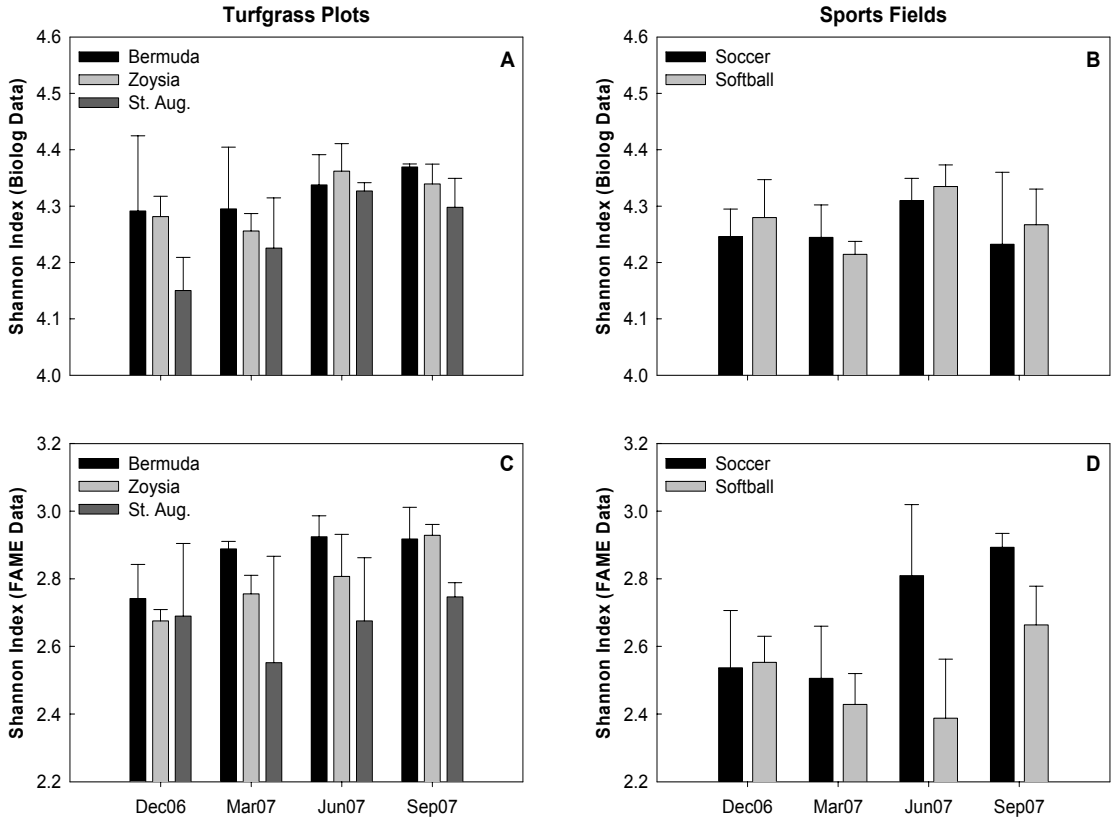

Fig. 26 – Shannon diversity indices for carbon source utilization (CSU) data from three turfgrass plots (A) and two sports fields (B), as well as for fatty acid methyl ester (FAME) data from both the turfgrass plots (C) and sports fields (D). Error bars represent the standard deviation of three replicate samples.

were no significant differences between the native and sand-based soils, or the turfgrasses and sports fields, based on CSU. Interestingly, comparison of diversity indices calculated from FAME profiles yielded no significant differences over time, which is different from the seasonal variation observed in the individual FAME sampling dates (Fig. 23). However, there was a significant difference between the Bermudagrass and softball field (Holm-Sidak, $p = 0.011$), with the Bermudagrass containing a greater diversity of FAMEs, indicating some uniqueness between the communities of the native soil and sand-based root zone.

Analysis of the CSU profile data with Shannon Index values by one-way ANOVA did not identify any significant differences between the communities in the native soil and sand-based root zones. The similarity of CSU profiles may indicate that regardless of soil or turf type, microbial communities utilized similar carbon substrates. The Biolog® GN2 plate inoculum was from the same dilution series used for the plate counting procedure, and as such may not have adequately represented the anaerobic microbial community in the native soil and sand-based root zones, which may have identified unique carbon utilization profiles to each location. The similarities between the soils may have also resulted from the fact that the Biolog plates utilize a fairly large assortment of bioavailable carbon substrates. Since heterotrophic organisms demonstrate an enormous flexibility with regards to what carbon sources they may use, similar CSU profiles might be expected in the native soil and sand-based root zones. We did, however, find significant differences between the individual sampling dates, specifically in June. The June sampling date indicated a higher level of diversity (i.e.,

more substrates used during the peak summer season) among CSU profiles than March and December, but not September, suggesting a gradual increase in the range of substrates used over time. This is in agreement with the population data, which also showed a general increase in microbial numbers over time. I was not able to identify any differences between the native soil and sand-based root zones, based on the diversity of CSU profiles, but I was able to detect a seasonal change (i.e., increase in CSU profile diversity over time) when comparing each sampling date.

The Shannon Index of the FAME profiles (Fig. 26) was also assessed with one-way ANOVA, to identify differences in community diversity between the turfgrasses and soils, over time. The microbial diversity as determined through fatty acid profiles was much higher with regards to the Bermudagrass plot than with the softball field, and this may be indicative of differences in the physico-chemical properties of the Boonville soil and sand-based root zones, or possibly of relative age of the two locations. However, I did not detect differences in microbial diversity, based on FAME, over time.

CONCLUSIONS

We were not able to identify any significant differences between the microbial populations from the native soil and sand-based root zones based on culture-based enumeration or carbon substrate utilization (CSU). The similarities between microbial populations for the "typical" lawn grasses and well-managed sports fields, indicate that microbial communities across an urban environment may not be that different with regards to population size and carbon substrate usage. However, microbial community composition, based on FAME profiles, can vary with respect to the native soil and the sand-based root zones, as well as with respect to turfgrass species and land use (i.e., lawns versus sports fields). This suggests that across the urban landscape unique microbial populations may develop and such changes warrant future investigation, as they may have an effect on management strategies to minimize off-site environmental impacts of an ever increasing turfgrass environment. Further analysis with molecular-based methods may reveal microbial populations specific to different land-use and management regimes.

SUMMARY

In summary, concentrations of DOC, DON, and DOP were determined in soil solutions under three warm-season turfgrasses grown on a native soil and in drainage water samples from under two sand-based sports fields. Soil solutions and drainage waters have the potential to move off site and into "natural" hydrological ecosystems, which could lead to the impairment of waterways, due to the increased potential for both eutrophication and pathogen survival (i.e., the introduction of nutrients that are available for microbial uptake), and as such, further research is needed. Home owners should be encouraged to have their soils analyzed for quantities of preexisting nitrogen, phosphorus, etc., to understand how much is already there, and how much needs to be added for adequate soil fertility. The high DON concentrations observed at the sports fields, during fertilization events, indicates that sports-field managers also need to be careful with fertilizer applications and mindful of the fact that in addition to fertilizer nutrients, dissolved organic nutrients may also move through the soil profile and into the drainage system. Identifying a mechanism to recycle at least some of the drainage water may alleviate this problem for sports field managers and may have the potential to lower costs over the long run with fewer fertilization applications. However, the potential for accumulation of sodium (Na) and other salts with water reuse may mitigate against this practice, especially where municipal water supplies are high in Na as they are in College Station, TX.

We were not able to identify any significant differences between the microbial populations from the native soil and sand-based root zones based on culture-based

enumeration or carbon substrate utilization (CSU). The similarities between microbial populations for the "typical" lawn grasses and well-managed sports fields, indicate that microbial communities across an urban environment may not be that different with regards to population size and carbon substrate usage. However, microbial community composition, based on FAME profiles, can vary with respect to the native soil and the sand-based root zones, as well as with respect to turfgrass species and land use (i.e., lawns versus sports fields). This suggests that across the urban landscape unique microbial populations may develop and such changes warrant future investigation, as they may have an effect on management strategies to minimize off-site environmental impacts of an ever increasing turfgrass environment. Further analysis with molecular-based methods may reveal microbial populations specific to different land-use and management regimes.

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APPENDIX

Appendix 1a – Fertilization rates (lbs/1,000ft^2; 34-0-0 Ammonium Nitrate)*

St. Augustinegrass

←North South→

1.0	3.0	2.0	0.0	0.5
0.0	0.5	3.0	2.0	1.0
0.5	2.0	1.0	0.0	3.0
3.0	1.0	0.0	2.0	0.5

- cells represent the location of a soil solution extraction well.

- cells represent the location of soil core extractions.

*Fertilizer was broadcast using a spinner walk-behind spreader with 2 passes and 100 overlap per pass.

**Appendix 1b – Fertilization rates (lbs/1,000ft^2; 34-0-0 Ammonium Nitrate)*

Zoysiagrass

←North South→

0.0	3.0	2.0	0.5	1.0
2.0	1.0	3.0	0.0	0.5
1.0	3.0	2.0	0.5	0.0
3.0	0.0	1.0	2.0	0.5

- cells represent the location of a soil solution extraction well.

- cells represent the location of soil core extractions.

*Fertilizer was broadcast using a spinner walk-behind spreader with 2 passes and 100 overlap per pass.

Appendix 2a – Fertilization of the Texas A&M soccer field as reported by the sports field manager (Craig Potts)[1]

Date	Ratio	Rate of Fertilization
2/14/2005	16-0-14	0.25lbs N/1,000ft^2
4/1/2005	16-0-14	0.5lbs N/1,000ft^2
4/15/2005	16-0-14	0.5lbs N/1,000ft^2
4/21/2005	21-0-0	1.0lbs N/1,000ft^2
5/4/2005	21-0-0	0.5lbs N/1,000ft^2
5/18/2005	21-0-0	1.0lbs N/1,000ft^2
6/2/2005	21-0-0	1.0lbs N/1,000ft^2
6/16/2005	21-0-0	1.0lbs N/1,000ft^2
6/30/2005	21-0-0	1.0lbs N/1,000ft^2
7/6/2005	21-0-0	0.5lbs N/1,000ft^2
7/12/2005	21-0-0	0.5lbs N/1,000ft^2
7/12/2005	0-0-22	0.5lbs K/1,000ft^2
7/12/2005	Step Micro Package	4 bags/2.2acres
7/23/2005	15-5-10	1.0lbs N/1,000ft^2
8/2/2005[2]	12-0-0	0.02lbs N/1,000ft^2
8/3/2005	0-0-5	0.012lbs K/1,000ft^2
8/6/2005	15-5-10	0.75lbs N/1,000ft^2
8/9/2005[3]	20-0-4 +1	4.0oz/1,000ft^2
8/17/2005	21-0-0	0.5lbs N/1,000ft^2
8/17/2005	0-0-50	1.0lbs K/1,000ft^2
8/20/2005	Gypsum	1.0ton/acre
8/22/2005	Iron Max	5oz/1,000ft^2
8/23/2005	Perk Up	1oz/1,000ft^2
8/23/2005[3]	20-0-4 +1	3oz/1,000ft^2
2/6/2007	15-0-15	0.5lbs N/1,000ft^2
2/20/2007[2]	12-0-0	0.09lbs N/1,000ft^2
2/20/2007[4]	N-Iron	8oz/1,000ft^2
4/25/2007	15-0-15	1.0lbs N/1,000ft^2
4/25/2007	Sulfur	1.5lbs S/1,000ft^2
5/2/2007[2]	12-0-0	0.06lbs N/1,000ft^2
5/2/2007[4]	N-Iron	6.5oz/1,000ft^2
5/11/2007	15-0-15	0.5lbs N/1,000ft^2
5/18/2007	15-0-15	0.5lbs N/1,000ft^2
5/22/2007	10-3-5	0.05lbs N/1,000ft^2
5/22/2007	5-0-7	0.01lbs N/1,000ft^2
5/31/2007	10-0-10	0.05lbs N/1,000ft^2
5/31/2007	21-0-18	0.38lbs N/1,000ft^2
6/8/2007	18-0-9	0.5lbs N/1,000ft^2
6/8/2007	Sulfur	1.0lbs S/1,000ft^2
6/20/2007	18-0-9	0.38lbs N/1,000ft^2
6/29/2007	18-0-9	0.38lbs N/1,000ft^2
7/2/2007	0-0-22	1.0lbs K/1,000ft^2
7/10/2007	10-3-5	0.075lbs N/1,000ft^2

Appendix 2b – Fertilization of the Texas A&M softball field as reported by the sports field manager (Craig Potts)

Date	Ratio	Rate of Fertilization
4/5/2006	15-0-15	0.38lbs N/1,000ft^2
5/16/2006	15-0-15	0.5lbs N/1,000ft^2
6/1/2006	15-0-15	0.5lbs N/1,000ft^2
6/1/2006	Sulfur	1.0lbs S/1,000ft^2
6/27/2006	15-0-15	0.5lbs N/1,000ft^2
7/5/2006	Defense-Man & Turgor	2oz/1,000ft^2
7/7/2006	15-0-15	0.5lbs N/1,000ft^2
7/20/2006	20-0-4 +1	5oz/1,000ft^2
7/20/2006	Perk Up Calcium	1gal/acre
7/31/2006	15-0-0	0.2lbs N/1,000ft^2
8/4/2006	20-0-4 +1	6oz/1,000ft^2
8/4/2006	20-0-6	0.24lbs N/1,000ft^2
8/14/2006	15-0-15	0.5lbs N/1,000ft^2
8/17/2006	Sulfur	2.0lbs S/1,000ft^2
8/18/2006	15-0-15	0.5lbs N/1,000ft^2
10/6/2006	15-0-15	0.5lbs N/1,000ft^2
10/6/2006	Sulfur	1.0lbs S/1,000ft^2
2/11/2007	15-0-15	0.5lbs N/1,000ft^2
2/19/2007[2]	12-0-0	0.03lbs N/1,000ft^2
2/19/2007[4]	N-Iron	5.5oz/1,000ft^2
2/27/2007[2]	12-0-0	0.0616lbs N/1,000ft^2
2/27/2007[4]	N-Iron	5.5oz/1,000ft^2
5/14/2007	15-0-15	0.5lbs N/1,000ft^2
6/5/2007[2]	12-0-0	0.09lbs N/1,000ft^2
6/5/2007	0-0-28	0.08lbs K/1,000ft^2
6/13/2007	Gypsum	1ton/acre
6/14/2007	18-0-9	1.0lbs N/1,000ft^2
6/29/2007	18-0-9	1.0lbs N/1,000ft^2
7/2/2007	0-0-22	1.0lbs K/1,000ft^2
7/2/2007	18-0-9	0.5lbs N/1,000ft^2
7/12/2007	18-0-9	0.38lbs N/1,000ft^2

[1]Records of fertilization rates were not given for 2006 due to a clerical error. However, we were informed that fertilization rates for 2005 were similar to 2006.
[2]Largo Fertilizer was applied and consisted of: 12.0% Urea, 4.2% Sulfur, 6.0% Iron, 1.5% Manganese, and 1.0% Zinc.
[3]Carbon Power was applied and consisted of: 5.28% Ammonium, 5.40% Nitrate, 9.32% Urea, 4.00% Potash, and 1Mg Carbon.
[4]N-Iron was applied and consisted of: 4% Sulfur and 6% Iron.

Appendix 3 - Mean concentrations of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), dissolved organic phosphorus (DOP), and dissolved inorganic phosphorus (DIP) found in other literature.

Literature Cited and Land Use	Mean DOC mg L^{-1}	Literature Cited and Land Use	Mean DON mg L^{-1}	Mean DIN mg L^{-1}	Literature Cited and Land Use	Mean DOP mg L^{-1}	Mean DIP mg L^{-1}
Mertens et al., 2007 Bare Agriculture Field Germany	17.0 ± 10.6 (max = 92.5)	Christou et al., 2005 Citrus Orchards England/Greece	≈30	≈75	McDowell and Koopmans, 2006 Pasture Field New Zealand	0.39	0.1*
Liu and Sheu, 2003 Natural Hardwood Forest Taiwan	21.3 ± 11.9	Khalid et al., 2007 *Lolium perenne* England	≈3	≈60	McDowell and Sharpley, 2001 *Lolium perenne* Pennsylvania	0.13	0.02*
Khalid et al., 2007 *Lolium perenne* England	≈30.0	Kessel et al., 2009 Ryegrass/Maize Germany	2.6	18.6	Langlois et al., 2003 Forest Canada	0.20 ± 0.02	0.51 ± 0.01

*Calculated as dissolved reactive phosphorus

Appendix 4 - Biolog® GN2 Microplate Carbon Substrates (Biolog Inc., Hayward, CA 2001)

Well	A	B	C	D	E	F	G	H
1	Water	i-erythritol	D-melibiose	Acetic acid	p-hydroxy phenylacetic acid	Bromosuccinic acid	L-histidine	Urocanic acid
2	α-cyclodextrin	D-fructose	β-methyl D-glucoside	cis-aconitic acid	Itaconic acid	Succinamic acid	Hydroxy L-proline	Inosine
3	Dextrin	L-fucose	D-psicose	Citric acid	α-keto butyric acid	Glucuronamide	L-leucine	Uridine
4	Glycogen	D-galactose	D-raffinose	Formic acid	α-keto glutaric acid	L-alaninamide	L-ornithine	Thymidine
5	Tween 40	Gentiobiose	L-rhamnose	D-galactonic acid lactone	α-keto valeric acid	D-alanine	L-phenylalanine	Phenyl-ethylamine
6	Tween 80	α-D-glucose	D-sorbitol	D-galacturonic acid	D,L-lactic acid	L-alanine	L-proline	Putrescine
7	N-acetyl-D-galactosamine	m-inositol	Sucrose	D-gluconic acid	Malonic acid	L-alanyl-glycine	L-pyroglutamic acid	2-amino-ethanol
8	N-acetyl-D-glucosamine	α-D-lactose	D-trehalose	D-glucosaminic acid	Propionic acid	L-asparagine	D-serine	2,3-butanediol
9	Adonitol	Lactulose	Turanose	D-glucuronic acid	Quinic acid	L-aspartic acid	L-serine	Glycerol
10	L-arabinose	Maltose	Xylitol	α-hydroxy-butyric acid	D-saccharic acid	L-glutamic acid	L-threonine	D,L-α-glycerol phosphate
11	D-arabitol	D-mannitol	Pyruvic acid methyl ester	β-hydroxy-butyric acid	Sebacic acid	Glycyl-L-aspartic acid	D,L-carnitine	α-D-glucose-1-phosphate
12	D-cellobiose	D-mannose	Succinic acid mono-methyl-ester	γ-hydroxy-butyric acid	Succinic acid	Glycyl-L-glutamic acid	γ-amino butyric acid	D-glucose-6-phosphate

Appendix 5 - Individual Fatty Acids* Used to Create FAME Profiles From Whole-Soil Extracts

12:0	16:0	18:3 w6c	18:0 2OH	23:0
14:0 I	I 17:1 G	18:1 w9c	cis 9,10 epoxy 18:0	24:0
14:0	Sum In Feature 4	Sum In Feature 7	20:1 w9c	25:0
15:0 I	17:0 I	Sum In Feature 8	20:0	28:0
15:0 A	17:0 A	18:1 w5c	20:4 w6c	C25 N Alcohol
15:0	17:1 w8c	18:0	Sum In Feature 12	C23 Primary Alcohol
I 16:1 H	17:0 cyclo	19:1 (w 8?) Alcohol	21:0	13:0 I
16:0 I	17:0	Sum In Feature 11	C18 N Alcohol	20:3 w6c
16:1 w9c	16:1 2OH	19:2 w6c.	22:0	18:0 12OH
16:1 w7c	18:1 w9t Alcohol	19:1 w11c	24:1 w9c Nervonic	
16:1 w5c	18:2 w6c	19:0 cyclo c11-12	Unknown 22.267 "C"	

*Fatty Acid nomenclature consists of: number of carbon atoms, number of double bonds, and the location of the double bond from the mehtyl end of the molecule (w). Isomers are indicated by *c* for cis and *t* for trans. Prefixes and suffixes *I* and *A* denote iso- and anteiso-branched fatty acids. Cyclopropyl acids are indicated by *cyclo* .

VITA

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