EVALUATION OF TURFGRASS PRODUCTS IN SOIL: IMPACTS ON MICROBIAL COMMUNITIES AND RESPONSE TO SOIL TEMPERATURE AND MOISTURE EFFECTS

by

ALEXX A. DIERA

(Under the Direction of Mussie Y. Habteselassie)

ABSTRACT

Research was conducted at a golf course in Johns Creek, GA in March to August 2017 to evaluate short-term effects of wetting agents, plant growth regulators, and *Trichoderma atroviride* on microbial abundance and function using quantitative polymerase chain reaction, phosphatase, urease, and soil respiration assays. In the putting green, bacteria and AOB exhibited sensitivity to wetting agents and *T. atroviride*, and urease activity was stimulated by the Cascade Plus and Duplex. In the fairway, bacteria exhibited transient proliferation to PrimoMaxx, and urease activity was depressed by Cutless MEC. Phosphatase activity was unaffected by treatments. Soil respiration indicated transient microbial responses to treatments. Laboratory research was performed to observe soil temperature and moisture effects on *T. atroviride* over 57 d. Growth rates were comparable among temperatures, but negatively correlated with soil moisture. *T. atroviride* may be most suited to establish itself in soil when applied in early to late spring in Georgia.

INDEX WORDS: microbial abundance, turfgrass, golf course, wetting agent, plant growth regulator, *Trichoderma atroviride*, ammonia-oxidizing archaea, ammonia-oxidizing bacteria, soil bacteria, soil fungi, phosphatase, urease, putting green, fairway, quantitative polymerase chain reaction, qPCR, soil moisture, soil temperature

EVALUATION OF TURFGRASS PRODUCTS IN SOIL: IMPACTS ON MICROBIAL COMMUNITIES AND RESPONSE TO SOIL TEMPERATURE AND MOISTURE EFFECTS

by

ALEXX A. DIERA

BS, University of North Carolina Asheville, 2014

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2018

EVALUATION OF TURFGRASS PRODUCTS IN SOIL: IMPACTS ON MICROBIAL COMMUNITIES AND RESPONSE TO SOIL TEMPERATURE AND MOISTURE EFFECTS

by

ALEXX A. DIERA

Major Professor:	Mussie Y. Habteselassie
Committee:	Miguel L. Cabrera
	Paul L. Raymer

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2018

ACKNOWLEDGEMENTS

I want to thank Dr. Mussie Habteselassie, my mentor and advisor, for his unequivocal support and guidance throughout my research and work at the University of Georgia. I would like to extend a special mention to Dr. Miguel Cabrera and Dr. Paul Raymer for their encouragement and insight towards improving this manuscript.

I want to extend my gratitude to Wendy Griffin for the tremendous support throughout the duration of my graduate program. From early morning samplings in the field to expert analysis in the lab, her friendship and expertise has been invaluable. You are an extraordinary friend and colleague. Thank you for your pragmatism and encouragement.

Thank you to Mark Hoban, the superintendent of Rivermont Golf Club, for providing the space, time, and tools to support the field component of this research. A special mention to Dr. Viktor Tishchenko for providing dataloggers and guidance on collecting soil temperature and moisture data in the field.

To my mother and sister, Susan and Aneka: thank you for love and boundless support.

Finally, I want to express my deepest appreciation for the unofficial Soil Microbiology department at UGA Griffin: I will always cherish afternoon tea with my most respected colleagues.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Turfgrasses are estimated to cover nearly 40% of the total surface area used for urban development in the contiguous 48 states (Milesli et al., 2005). Turf pervades the landscape of the United States as lawns, roadsides, golf courses, athletic fields, parks, and other amenities. Although often unnoticed and taken for granted, such vast coverage across the country presents innumerable opportunities to actualize the full benefits that turfgrasses present to the environment, society, and economy: improving soil quality, filtering air, mitigating runoff, tempering the impact of irradiance, providing spaces for health and wellness, and adding $58 billion to the U.S. economy from turf and related industries (Strandberg et al., 2012; Stier et al., 2013; Christians et al., 2016; Haydu et al., 2006).

Turf systems require diligent maintenance, therefore, optimizing the efficient use of resources is critical to the industry. Golf course maintenance, alone, epitomizes central challenges involved in turf management today. Superintendents are expected to maintain high quality turf under longer growing seasons, extreme weather events, and increasing pressure to optimize resource use efficiency and reduce chemical fertilizer and pesticide inputs (Strandberg et al., 2012; Johnson et al., 2013; Breuninger et al., 2013; Stier et al., 2013). To mitigate some impacts of these stresses on turfgrasses, superintendents are incorporating organic and biological products like wetting agents, plant growth regulators, and microbial inoculants into their maintenance regimes (Christians et al., 2016; Reicher et al., 2013; Nelson and Craft, 1998).

Wetting agents are alcohol-based surfactants used to increase water filtration through the soil profile and reduce water repellency caused by localized dry spots (LDS) primarily in sandy turf soils (Kostka 2000; Barton and Colmer, 2011). PGRs are applied to promote turfgrass root production by inhibiting shoot growth for variety of reasons including improving turf density and mitigating stress from heat, drought, shade, and mowing (Stier et al., 1999; Reicher et al., 2013; Lickfeldt et al., 2001; Zhang et al., 2003). Microbial inoculants are used to address a variety of issues including disease control, plant nutrient uptake, and thatch-mat layer reduction (Nelson and Craft, 1998; Smith and Collins, 2008; Gaussoin et al., 2013; Christians et al., 2016). Little is known, however, about how wetting agents, plant growth regulators, and microbial inoculants affect turfgrass native turfgrass-soil microbial communities.

Like other soil systems, microorganisms in turf profiles provide important ecosystem functions centered on organic matter decomposition, nutrient cycling, and synthetic chemical degradation. Microbial decomposers are primarily responsible for breaking down organic matter into plant-available carbon, nitrogen, and other nutrients essential to plant health (Schmidt et al., 2013; Gaussoin et al., 2013; van der Heijden et al., 2008; Barrios, 2007). Organic matter turnover is particularly important in turf care to reduce the thatch build-up on the surface of turfgrasses that can limit root growth, prevent air and water flow through the soil, and increase turf's vulnerability to diseases (Gaussoin et al., 2013; Christians et al., 2016). Soil microbial activity can also indirectly minimize the loss of synthetic fertilizers, pesticides, and pollutants into the environment (Arias-Estévez et al., 2008; Gaussoin et al., 2013; Reedlich et al., 2017). Other beneficial services microorganisms in soils perform include forming mutualistic relationships with grass roots in order to enhance nutrient acquisition, providing antibiotic protection against pathogens, and improving plant survivability under abiotic stresses from the

environment such as heat, drought, and soil compaction (Powell and Klironomos, 2007; Barrios, 2007; Christians et al., 2016; Gaussoin et al., 2013).

Purpose of study

Soil microbial communities in turf systems are vital for maintaining healthy swards of grass, but the impact of wetting agents, plant growth regulators, and microbial inoculants is largely unknown. As interest in soil microbiology continues to increase in the turf industry, scientific data is needed to evaluate the effects of these products on soil microbial communities and their ecosystem functions.

The primary objective of this research is to evaluate the short-term impact of select wetting agents, plant growth regulators, and the microbial inoculant *Trichoderma atroviride* on the abundance and activities of microbial communities in turfgrass-soil systems. The second objective is to estimate optimal soil temperature and moisture conditions for a *T. atroviride* inoculant in a lab study using a sandy Georgia soil.

REFERENCES

Arias-Estévez, M., E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J. Mejuto, and L. García-Río. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agr. Ecosyst. Environ. 123:247–260. doi:10.1016/j.agee.2007.07.011

Barrios, E. 2007. Soil biota, ecosystem services and land productivity. Ecological Economics. 64:269–285. doi:10.1016/j.ecolecon.2007.03.004

Barton, L., and T.D. Colmer. 2011. Ameliorating water repellency under turfgrass of contrasting soil organic matter content: Effect of wetting agent formulation and application frequency. Agric. Water Manag. 99:1–7. doi:10.1016/j.agwat.2011.08.009

Bonkowski, M., C. Villenave, and B. Griffiths. 2009. Rhizosphere fauna: The functional and structural diversity of intimate interactions of soil fauna with plant roots. Plant Soil. 321:213–233.

Breuninger, J.M., M.S. Welterlen, B.J. Augustin, V. Cline, and K. Morris. 2013. The turfgrass industry. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 37–103.

Christians, N.E., A.J. Patton, and Q.D. Law. 2017. Fundamentals of turfgrass management. 5th ed. John Wiley & Sons, Hoboken, NJ.

Gaussoin, R.E., W.L. Berndt, C.A. Dockrell, and R.A. Drijber. 2013. Characterization, development, and management of organic matter in turfgrass systems. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 425–456.

Haydu, J.J., Hall, C.R., and A.W. Hodges. 2006. Economic impacts of the turfgrass and lawncare industry in the United States. Univ. of Florida, Food and Resource Econ. Dep. http://edis.ifas.ufl.edu/pdffiles/FE/FE63200.pdf (accessed 24 Mar. 2018).

Johnson, P.G., F.S. Rossi, and B.P. Horgan. 2013. Sustainable turfgrass management in an increasingly urbanized world. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 1007–1028.

Kostka, S.J. 2000. Amelioration of water repellency in highly managed soils and the enhancement of turfgrass performance through the systematic application of surfactants. J. Hydrol. 231–232:359–368.

Lickfeldt, D.W., D.S. Gardner, B.E. Branham, and T.B. Voight. 2001. Implications of repeated trinexapac-ethyl applications on Kentucky bluegrass. Agron. J. 93:1164–1168.

Milesi, C., S.W. Running, C.D. Elvidge, J.B. Dietz, B.T. Tuttle, and R.R. Nemani. 2005. Mapping and modeling the biogeochemical cycling of turf grasses in the United States. Environ. Manage. 36:426–438.

Nelson, E.B., and C.M. Craft. 2000. Microbial strategies for the biological control of turfgrass diseases. In: J.M. Clark and M.P. Kenna, editors, Fate and management of turfgrass chemicals. ACS Symp. Ser. American Chemical Society, Washington, D.C. p. 342–352.

Powell, J., and J. Klironomos. 2008. The ecology of plant-microbial mutualisms. In: E.A. Paul, editor. 2008. Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 257–281.

Reedlich, L.M., M.D. Millican, and P.L. Koch. 2017. Temperature impacts on soil microbial communities and potential implications for the biodegradation of turfgrass pesticides. J. Environ. Qual. 46:490–497. doi:10.2134/jeq2017.02.0067

Reicher, Z.J., P.H. Dernoeden, and D.S. Richmond. 2013. Insecticides, fungicides, herbicides, and growth regulators used in turfgrass systems. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 891–932.

Schmidt, M.W.I., Torn, M.S., S. Abiven, T. Dittmar, G. Guggenberger, I.A. Janssens, M. Kleber, I. Kögel-Knabner, J. Lehmann, D.A.C. Manning, P. Nannipieri, D.P. Rasse, S. Weiner, and S.E. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature. 478:49–56. doi:10.1038/nature10386

Smith, J.L., and H.P. Collins. 2008. Management of organisms and their processes in soils. In: E.A. Paul, editor. 2008. Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 471–502.

Stier, J.C., J.N. Rogers, III, J.R. Crum, and P.E. Rieke. 1999. Flurprimidol effects on Kentucky bluegrass under reduced irradiance. Crop Sci. 39:1423–1430.

Stier, J.C., K. Steinke, E.H. Ervin, F.R. Higginson, and P.E. McMaugh. 2013. Turfgrass benefits and issues. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 105–145.

Strandberg, M., K. Blombäck, A.M. Dahl Jensen, and J.W. Knox. 2012. Priorities for sustainable turfgrass management: A research and industry prospective. Acta Agric. Scand., Sect. B. 62:3–9.

van der Heijden, M.G.A., R.D. Bardgett, and N.M. van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296–310. doi:10.1111/j.1461-0248.2007.01139.x

Zhang, X.Z., E.H. Ervin, and R.E. Schmidt. 2003. Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. Crop Sci. 43:952–956.

CHAPTER 2

LITERATURE REVIEW

Golf course soil systems

Smooth, lush, green ground cover and wide, open spaces characterize the most desirable golf courses around the world. Like all turfgrass systems, golf courses are inherently anthropogenic, highly-managed landscapes. Because established turfgrasses are perennial systems requiring three times more water than the United States' most irrigated crop corn (Falk, 1976; Milesi et al., 2005), turf ecosystems stand apart from other managed environs such as agricultural fields or grasslands. This high-volume water consumption makes optimizing management practices the key to maintaining well-irrigated and fertilized golf courses while curtailing the economic and ecological costs to do so. Cultivating turf swards with vigor and rooting capability is a primary goal in golf course maintenance. Hence, fostering soil health is essential for promoting long-term, sustainable turf quality (Bigelow and Soldat, 2013). According to Doran and Zeiss (2000), soil health is defined as "the capacity of soil to function as a vital living system to sustain biological productivity, promote environmental quality, and maintain plant and animal health." The soil matrix is a complex ecosystem consisting of minerals, water, air, flora, fauna, organic material, and a myriad of physical, chemical, and biological interactions that affect turf growth and quality (Voroney, 2007). Biological processes such as decomposition and nutrient cycling are driven by microbial activity that directly impact soil health (Gaussoin et al., 2013). The relationships among management intensity, thatch formation, and rhizosphere characteristic of golf course soils are

central to building a framework towards a clearer understanding of soil microorganisms in these systems.

Golf courses comprise playing surfaces that have distinct management intensity levels from one another. Each specific surface management affects microbial activity differently. The more intensely managed systems include putting greens and teeing grounds which are constructed with sand-based root zones and drainage systems to improve nutrient retention, minimize soil compaction, and ensure playable, uniform turf surfaces (Bigelow and Soldat, 2013; Christians et al., 2016). These spaces also tend to receive more frequent applications of fertilizers, wetting agents, and other turf care products in contrast to the fairways (Bartlett et al., 2007; 2009). Fairways are also managed to accommodate foot traffic and playability, but the management approach varies widely based on native soil conditions. For example, a mixture of sand and peat moss known as topdressing is typically applied to finer soil textures to increase soil porosity and promote water filtration. Coarser soils may be amended with peat moss or other organic substrates to improve nutrient retention (Bigelow and Soldat, 2013). While the effects of differing levels of management intensity in golf courses have not been widely studied, Bartlett et al. (2009) analyzed biomass carbon (C) and observed smaller community sizes correlated to highly managed soils. This study also detected a correlation between sand content and phenotypic variation among soil microbial community structures via phospholipid fatty acid (PFLA) analysis due to larger pore space and resource access in the putting greens and teeing grounds. Furthermore, the microbial communities among managed turf formed quickly and were similar to one another, but unique to communities in other types of land use (Bartlett et al., 2007; 2009). Irrigation practices appear to be the more influential component of golf course maintenance on soil microbial communities than turfgrass management (Mu and Carroll, 2013).

Thatch formation common to turfgrass soils are also an important management consideration in golf course soils directly related to microbial activity. Thatch is typically a compact layer formed between the turf canopy and soil surface as a result of intermingling root tissues and decomposing organic residues. Thatch is directly mediated by soil microorganisms breaking down organic material from dead root tissues and other organisms in various stages of life and decay (Gaussoin et al., 2013). Modest-sized layers of thatch (approximately 1.27 cm) can provide bounce to the soil surface, a habitat for beneficial micro- and macrofauna, and a barrier between chemical inputs and groundwater. However, larger thatch layers can become problematic by limiting root growth, preventing air and water flow through the soil matrix, and promoting pathogenic microbial activity (Christians et al., 2016). Thatch development is often managed in golf courses by forming a "thatch-mat" layer by intermixing topdressing to dilute negative impacts of excess thatch (Christians et al., 2016).

The rhizosphere formed in golf course soils are similar to those in other turfgrass systems and are the primary media for microbial activity, root growth, nutrient uptake, and water flow. Turfgrass soils are typically highly disturbed, dense, coarse-textured, and experience the consequences of soil compaction due to traffic. The reduced pore space and modified pore size distribution negatively affect gas exchange and water movement (Bigelow and Soldat, 2013). Soil air and water are components of the soil matrix that affect both turf growth and microbial activity. Limited pore space restricts turfgrass roots' access to water and nutrients, and the availability of water and air throughout the soil profile is critical to nutrient and habitat access for microorganisms (Bigelow and Soldat, 2013; Voroney, 2007).

The differing levels of management intensities, thatch formation, and root-zones in golf courses present a broad framework of the habitat that soil microbial communities live

in. Intensive management and high disturbance in golf course soils promote the establishment of specific, robust microbial communities.

Wetting agents, growth regulators, and Trichoderma atroviride

Golf course management regimes address a variety of turfgrass stresses to maintain dense, green turfgrass. Turfgrass can be subjected to stresses from a variety of factors including thatch accumulation, heavy rainfall, drought, heat, and disease (Christians et al., 2016). Wetting agents, plant growth regulators (PGRs), and microbial inoculants are among the many products sold on the market to improve turf health and resiliency. These products are commonly incorporated into golf courage management programs, but the direct impact of treatment applications used to maintain golf courses on their soil microbial communities is limited.

One of the most common issues faced by golf course superintendents is the phenomenon of localized dry spots (LDS). LDS are the visual response of turfgrass to water repellency in primarily sand-based soil in which patches of turfgrass become necrotic. Water repellency in soil is caused by the polymerization of hydrophobic organic material around soil colloids after repeated cycles of wetting and drying (Kostka, 2000; Barton and Colmer, 2011). Few microorganisms can degrade this material (Hallett et al., 2001). Wetting agents are alcohol-based surfactants applied to soils as a preventive measure to prevent LDS and to ensure uniform soil moisture. The hydrophobic head of the active ingredient in wetting agents attach to soil colloids, while the hydrophilic tail allows water to pass through the soil matrix (Kostka, 2000). Research exploring the direct effects of wetting agents on turfgrass soil microorganisms have not been found in the literature. However, a related study has observed water repellency is promoted by fungi and suppressed by bacterial competition (Hallett et al., 2001). The efficacy of wetting agents in sandy soils have an inverse relationship with soil organic matter (SOM) content (Baton

and Colmer, 2011). Some wetting agents used in other fields including bioremediation, food safety, and agriculture have improved or hindered enzyme activity depending on their particular application (Singh et al., 2007; Kamiya et al., 2008). The impact of turf wetting agents on soil microbial activity is unknown.

Maintaining resilient, uniform turfgrass in the presence of shade, heat, drought, and other stresses presents another challenge to golf course superintendents. Active ingredients in PGRs mimic or influence growth hormones in turfgrass to promote root development while inhibiting shoot growth. PGRs are classified into two major categories based on their modes of action: Type I PGRs inhibit biological pathways involved in cell division, and Type II PGRs target processes related to the synthesis of gibberellin acids, naturally occurring compounds involved in plant development (Reicher et al., 2013). By promoting resource allocation towards root development underground, the ability of turfgrass to absorb water and nutrients mitigates their need to grow rapidly to compete for sunlight aboveground. Current research indicates that improved turf quality from PGRs bolsters turfgrass resilience against many stresses including shade, heat, irradiance, drought, compaction, mowing, and disease (Qian and Engelke, 1999; Zhang et al., 2003; Stier et al., 1999; Reicher et al., 2013; Lickfeldt et al., 2001). The relationship between PGRs and microbiology in golf course soils has not been extensively explored, but Feng et al. (2002) initially reported on the effects of PGR trinexapac-ethyl on turfgrass in warm and cool seasons in Alabama. The authors observed high levels of mycorrhizal infections in the trinexapac-ethyl plots and no differences in the structures of soil microbial communities via fatty acid methyl ester (FAME) analysis.

Preventing and mitigating turfgrass disease is another crucial component to golf course management. As public demand for lower chemical inputs increase, golf course superintendents

are turning towards biological control agents as alternative treatments to fungicides to ward off soil pathogens (Nelson 1997; Aamlid et al., 2012; Boulter et al., 2000). Common diseases in turfgrass in the southeastern United States include *Sclerotinia homoecarpa* (dollar spot), *Rhizoctonia solani* (brown patch), and *Pythium* spp. (Pythium root rot) among many others (Martinez et al., 2017). One biological control agent extensively explored in the literature is *Trichoderma* spp., a genus of predatory soil fungi that target plant pathogens (Harman et al., 2004; Harman, 2006; Verma et al., 2007). The primary mechanism of protection to turfgrass that *Trichoderma* spp. provide is through encoding antifungal enzymes like chitinases and β-1,3 glucanases into the root tissue, although the specific means and class of enzymes vary among plant hosts and *Trichoderma* strains (Harman et al., 2006). The absorption of these enzymes allows the turf to build varying levels of resistance to certain plant pathogens, including *S. homoecarpa*, *R. solani,* and *Pythium* spp. (Verma et al., 2007; Harman et al., 2004; 2006; Daryaei et al., 2016a; Daryaei et al., 2016b; Wong and McBeath, 1999; Lo et al., 1997).

Trichoderma atroviride Karsten CHS 861 is the active ingredient in PlantHelper (AmpPac Biotech, Fresno, CA) native to the sub-arctic region of Alaska. This strain of *T. atroviride* has been observed to directly parasitize plant pathogens without causing phytoxicity to host plants. Furthermore, *T. atroviride* Karsten CHS 861 grows within a temperature range of 4 to 33ºC which extends beyond several pathogen's temperature ranges such as *R. solani* (1 to 32ºC) and *Pythium* spp. (7 to 30ºC) (Wong and McBeath, 1999). One study testing the effects of PlantHelper on biological control activity could be found in the literature and observed total inhibition of *Phytophthora ramorum* (sudden oak death) on shrub leaves (Elliott et al., 2009). Other studies observed the biological control activity of different *T. atroviride* strains. Daryaei et al. (2016a, 2016b, 2016c, 2016d) observed shortened life spans of *T. atroviride*

LU132 in culture conditions, a conidium production cycle of 20 d, optimal germination and bioactivity in 30ºC, and ideal carbon-to-nitrogen ratio (C:N), water activity level (a_w) and pH as 5:1, 0.961, and 7.5 respectively. Savazzini et al. (2009) observed a two-week effect of *T. atroviride* SC1 on soil bacteria and fungi communities before the population size of the inoculant stabilized.

Wetting agents, PGRs, and *Trichoderma atroviride* are currently sold on the market to improve turf resilience in several ways. Present literature on the impact of these products on soil microorganisms is limited.

Microbiology in turfgrass soils

Soil microorganisms serve as one group of soil health indicators that can assist superintendents with management decisions to execute sustainable, cost-effective golf courses. Healthy soil ecosystems are characterized by their stable, resilient responses to stress and disturbance (Doran and Zeiss, 2000). Like other natural systems, soil microbial communities comprise of a variety of species that can be beneficial or antagonistic to turfgrass productivity. Healthy, robust soils foster the proliferation of diverse, favorable species, and the presence of some keystone species such as N-fixing bacteria *Rhizobium* spp. can serve as simple indicators of soil quality (Barrios, 2007; van Bruggen and Semenov, 2000; Fierer et al., 2007). Beneficial microorganisms also perform many essential ecosystem services, including organic matter turnover, nutrient recycling, disease suppression, modifying soil structure, plant nutrient acquisition, and chemical degradation (Doran and Zeiss, 2000; Barrios, 2007; Morgan et al., 2005; Veeh et al., 1996; Arias-Estévez et al., 2008; Reedlich et al., 2017). Microbial community composition, enzyme activity, and soil respiration serve as some simple soil health

indicators as demonstrated in past research connecting these elements of the soil habitat to turfgrass studies.

Microbial community abundance and diversity are among the most practical soil quality indicators, because they are influenced by different kinds of land management and plant cover (Doran and Zeiss, 2000; Yao et al., 2000). Biodiversity in soil systems are best evaluated at the microbial level by group, such as bacteria, fungi, ammonia-oxidizers, and many others, instead of species, because functional redundancy is expected from many soil microbial species (Barrios, 2007; van Bruggen and Semenov, 2000). Bacteria typically thrive in highly disturbed, nutrient-rich, early successional environments with low organic matter content. Fungi prefer late successional environments with less disturbance, low nutrient availability, and high organic matter content. These two types of environments determine whether nutrients will be lost quickly or recycled in a closed system respectively (Heijden et al., 2008).

Ammonia-oxidizing archaea (AOA) and bacteria (AOB) are two critical microorganisms involved in autotrophic nitrification, a fundamental process involved in the global N cycle. In autotrophic nitrification, AOA and AOB oxidize ammonia (NH_3) into hydroxylamine (NH_2OH) using the enzyme ammonia monooxygenase. NH_2OH is then transformed into nitrite (NO_2^-) by nitrite-oxidizing prokaryotes (Hatzenpichler, 2012; Norton and Stark, 2011; Norton, 2011). In soil systems, NO_2^- is often oxidized quickly back to water-soluble NO_3^- by nitrite oxdizers (Robertson and Groffman, 2007). AOA compared to AOB are oligotrophic microorganisms with robust cellular structures can survive under nutrient-, oxygen-depleted conditions and have been detected at 30ºC, the maximum temperature of survival for most microorganisms (Hatzenpichler, 2012). AOB are less populous than AOA, but some species have also been observed in extreme environments (Norton, 2011). As direct ammonia-oxidizing competitors, the availability of NH_4^+

and niche distribution determine the distribution of AOA and AOB in a given environment (Norton and Stark, 2011; Wessén and Hallin, 2011). Both ammonia-oxidizing groups are ubiquitous around in the world and serve as important indicators of N cycling in soil systems (Hatzenpichler, 2012; Norton, 2011; Wessén and Hallin, 2011; Wyngaard et al., 2016).

Another useful soil quality indicator is microbial enzyme activity to understand the functions within soil microbial communities (Nannipieri et al., 2002). Microbial extracellular enzymes involved in nutrient recycling remain present in soil after the microorganisms have decayed (Burns et al., 2013). Measuring soil enzymatic activity can elucidate the potential activity of nutrient turnover and other microbial activity of interest in a soil (Nannipieri et al., 2002; Burns et al., 2013). Extracellular enzymes such as phosphatase and urease are produced as a means to obtain organically-bound phosphorus (P) and N (Sinsabaugh et al., 2002). Most of these and other extracellular enzymes stabilize and may remain active when they bind to soil colloid surfaces, humic acids, and particulate organic matter. These complexed extracellular enzymes serve the soil microbial community by signaling changes in nutrient availability and degrading organic material when the community is stressed (Burns et al., 2013).

Soil respiration serves as another soil quality indicator to estimate decomposition rates of SOM based on rates via carbon dioxide (CO_2) evolution (Kandeler, 2007). SOM consists of humic substances, plant, animal, and microbial biomass at every stage of life and decay and is the largest terrestrial source of CO_2 (Kandeler, 2007; Schlesinger and Andrews, 2000). The stability of SOM is dependent on biological and environmental factors, particularly by increased microbial populations or activity (Schmidt et al., 2011; Kuzyakov et al., 2000). Observing SOM turnover provides insight into the flow of energy and nutrients into a soil food web system which

inevitably contributes mineralized nutrients to plants, stabilizes soil structure, and improves water retention, drainage, and cation exchange capacity (Barrios, 2007).

Past studies have examined the relationships between turfgrass systems and soil quality using microbial communities, enzyme assays, and soil respiration as indicators. Mueller and Kussow (2005) observed that biostimulant products that included materials such as bacterial and fungal inoculums, yucca, seaweed extract, and several others did not affect soil microbial enzyme activity in a putting green, but the authors observed other factors contributed to a decline in bacteria populations. The community composition of bacteria and archaea populations observed in a putting green soil correlated to seasonal changes over a 1-yr study, although some data suggested other influences on population fluctuations (Beirn et al., 2017). However, high temperatures (12 to 34ºC) simulated to reflect heat stress promoted the ability of soil microorganisms in a turfgrass to decompose organic material (Dell et al. 2012). The diversity and richness of AOB populations were not affected by turfgrass management practices, although the authors suggested NH_3 or SOM influenced the restructuring observed in the AOB community (Dell et al., 2008). Ye et al. (2009) observed comparable metabolic diversity between turfgrass and forest soils compared to pasture fields. Shi et al. (2006) observed a positive correlation between enzymes associated with humification and oxidation (glucosidase and phenol oxidase) and turf age. The rates of soil respiration observed in northern Colorado semi-arid soils were highest in urban lawns compared to three other land use types (Kaye et al., 2005). Over a 40-yr study in New Zealand, intensively-managed portions of a putting green also did not sequester soil C, although, interestingly, C sequestration increased 50% in undisturbed parts of the green (Huh et al., 2008).

Soil health indicators like microbial community composition, enzyme activity, and soil respiration have been used in recent turfgrass studies. However, the limited research available between soil microbiology and golf course management prompts infinite questions about the connections among the innumerable aspects of both respective fields.

REFERENCES

Aamlid, T.S., G. Thorvaldsson, F. Enger, and T. Pettersen. 2012. Turfgrass species and varieties for integrated pest management of Scandinavian putting greens. Acta Agric. Scand., Sect. B. 62:10–23.

Arias-Estévez, M., E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J. Mejuto, and L. García-Río. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agr. Ecosyst. Environ. 123:247–260. doi:10.1016/j.agee.2007.07.011

Barrios, E. 2007. Soil biota, ecosystem services and land productivity. Ecol. Econ. 64:269–285. doi:10.1016/j.ecolecon.2007.03.004

Bartlett, M.D., I.T. James, J.A. Harris, and K. Ritz. 2007. Interactions between microbial community structure and the soil environment found on golf courses. Soil Biol. Biochem. 39:1533–1541. doi:10.1016/j.soilbio.2007.01.003

Bartlett, M.D., I.T. James, J.A. Harris, and K. Ritz. 2009. Microbiological profiles of golf course soils. Int. Turfgrass Soc. Res. J. 11:949–956.

Barton, L., and T.D. Colmer. 2011. Ameliorating water repellency under turfgrass of contrasting soil organic matter content: Effect of wetting agent formulation and application frequency. Agric. Water Manag. 99:1–7. doi:10.1016/j.agwat.2011.08.009

Beirn, L.A., J.W. Hempfling, C.J. Schmid, J.A. Murphy, B.B. Clarke, and J.A. Crouch. 2017. Differences among soil-inhabiting microbial communities in *Poa annua* turf throughout the growing season. Crop Sci. 57:S-262–S-273. doi:10.2135/cropsci2016.06.0463

Bigelow, C.A., and D.J. Soldat. 2013. Turfgrass root zones: Management, construction methods, amendment characterization, and use. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 383–423.

Boulter, J.J., G.J. Boland, and J.T. Trevors. 2000. Compost: A study of the development process and end-product potential for suppression of turfgrass disease. World J. Microb. Biot. 16:115–134.

Breuninger, J.M., M.S. Welterlen, B.J. Augustin, V. Cline, and K. Morris. 2013. The turfgrass industry. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 37–103.

Burns, R.G., J.L. DeForest, J. Marxsen, R.L. Sinsabaugh, M.E. Stromberger, M.D. Wallenstein, M.N. Weintraub, and A. Zoppini. 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem. 58:216-234. doi:10.1016/j.soilbio.2012.11.009

Christians, N.E., A.J. Patton, and Q.D. Law. 2017. Fundamentals of turfgrass management. 5th ed. John Wiley & Sons, Hoboken, NJ.

Crouch, J.A., Z. Carter, and T. Yao. 2017. Soil microbiota variation in perennial ryegrass ruft and Festuca elata turf using illumina sequencing. Res. J. Biotechnol. 12:60–68.

Daryaei, A., E.E. Jones, H. Ghazalibiglar, T.R. Glare, and R.E. Falloon. 2016a. Culturing conditions affect biological control activity of *Trichoderma atroviride* against

Rhizoctonia solani in ryegrass. Journal of Applied Microbiology. 121:461-472. doi:10.1111/jam.13163

Daryaei, A., E.E. Jones, H. Ghazalibiglar, T.R. Glare, and R.E. Falloon. 2016b. Effects of temperature, light and incubation period on production, germination and bioactivity of *Trichoderma atroviride*. Journal of Applied Microbiology. 120:999-1009.

Daryaei, A., E.E. Jones, T.R. Glare, and R.E. Falloon. 2016c. Nutrient amendments affect *Trichoderma atroviride* conidium production, germination and bioactivity. Biological Control. 93:8-14. doi:10.1016/j.biocontrol.2015.11.003

Daryaei, A., E.E. Jones, T.R. Glare, and R.E. Falloon. 2016d. pH and water activity in culture media affect biological control activity of *Trichoderma atroviride* against *Rhizoctonia solani*. Biological Control. 92:24-30. doi:10.1016/j.biocontrol.2015.09.001

Dell, E.A., D. Bowman, T. Rufty, and W. Shi. 2008. Intensive management affects composition of betaproteobacterial ammonia oxidizers in turfgrass systems. Microb. Ecol. 56:178–190.

Dell, E.A., D.S. Carley, T. Rufty, and W. Shi. 2012. Heat stress and N fertilization affect soil microbial and enzyme activities in the creeping bentgrass (*Agrostis stolonifera* L.) rhizosphere. Appl. Soil Ecol. 56:19–26.

Doran, J.W., and M.R. Zeiss. 2000. Soil health and sustainability: Managing the biotic component of soil quality. Appl. Soil Ecol. 15:3–11.

Elliott, M., S.F. Shamoun, G. Sumampong, D. James, S. Masri and A. Varga. 2009. Evaluation of several commercial biocontrol products on European and North American populations of *Phytophthora ramorum*. Biocontrol Sci. Techol. 19:1007-1021, doi:10.1080/09583150903243870

Fierer, N., J.A. Jackson, R. Vilgalys, and R.B. Jackson. 2011. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71:4117-4120.

Fierer, N., M.A. Bradford, and R.B. Jackson. 2007. Toward an ecological classification of soil bacteria. Ecology 88:1354–1364.

Gaussoin, R.E., W.L. Berndt, C.A. Dockrell, and R.A. Drijber. 2013. Characterization, development, and management of organic matter in turfgrass systems. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 425–456.

Hallett, P.D., K. Ritz, and R.E. Wheatley. 2001. Microbial derived water repellency in golf course soil. Int. Turfgrass Soc. Res. J. 9:518–524.

Harman, G.E. 2006. Overview mechanisms and uses of *Trichoderma* spp. Phytopathology. 96:190-194. doi:10.1094/PHYTO-96-0190

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet, and M. Lorito. 2004. *Trichoderma* species—Opportunistic, avirulant plant symbionts. Nat. Rev. 2:43–56. doi:10.1038/nrmicro797

Hatzenpichler, R. 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. Applied and Environ. Microbiol. 78:7501-7510. doi:10.1128/AEM.01960-12

Huh, K.Y., M. Deurer, S. Sivakumaran, K. McAuliffe, and N.S. Bolan. 2008. Carbon sequestration in urban landscapes: The example of a turfgrass system in New Zealand. Aust. J. Soil Res. 46:610–616.

Kamiya, N., M. Inoue, M. Goto, N. Nakamura, Y. Naruta. 2000. Catalytic and structural properties of surfactant-horseradish peroxidase complex in organic media. Biotechnol. Prog. 16:52-58.

Kandeler, E. 2007. Physiological and biochemical methods for studying soil biota and their function. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 25-49.

Kaye, J.P., R.L. McCulley, and I.C. Burke. 2005. Carbon fluxes, nitrogen cycling, and soil microbial communities in adjacent urban, native and agricultural ecosystems. Glob. Change Biol. 11:575–587. doi:10.1111/j.1365-2486.2005.00921.x

Kostka, S.J. 2000. Amelioration of water repellency in highly managed soils and the enhancement of turfgrass performance through the systematic application of surfactants. J. Hydrol. 231–232:359–368.

Kuramae, E.E., E. Yergeau, L.C. Wong, A.S. Pijl, J.A. van Veen, and G.A. Kowalchuk. 2010. Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS Microbiol. Ecol. 79:12–24. doi: 10.1111/j.1574-6941.2011.01192.x

Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32:1485–1498.

Lickfeldt, D.W., D.S. Gardner, B.E. Branham, and T.B. Voight. 2001. Implications of repeated trinexapac-ethyl applications on Kentucky bluegrass. Agron. J. 93:1164–1168.

Lo, C.-T., E.B. Nelson, and G.E. Harman. 1996. Biological control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum*. Plant Dis. 80:736-741.

Martinez, A., M. Pearce, and L. Burpee. 2017. Turfgrass diseases in Georgia: Identification and control. Univ. Ga. Ext.

https://secure.caes.uga.edu/extension/publications/files/pdf/B%201233_7.PDF (accessed 14 May 2018).

Morgan, J.A.W., G.D. Bending, and P.J. White. 2005. Biological costs and benefits to plant-microbe interactions in the rhizosphere. J. Exp. Bot. 56:1729–1739.

Mu, Y., and M.J. Carroll. 2013. Thatch and soil microbial activity in recently cultivated turfgrass. Int. Turfgrass Soc. Res. J. 12:567–574.

Mueller, S.R., and W.R. Kussow. 2005. Biostimulant influences on turfgrass microbial communities and creeping bentgrass putting green quality. HortScience. 40:1904-1910.

Nannipieri, P., E. Kandeler, and P. Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. In: R.G. Burns and R.P. Dick, editors, Enzymes in the environment: Activity, ecology, and applications. Marcel Dekker, New York, NY. p. 1-33.

Nelson, E.B. 1997. Microbial inoculants for the control of turfgrass diseases. Int. Turfgrass Soc. Res. J. 8:791–811.

Norton, J.M. 2011. Diversity and environmental, distribution of ammonia-oxidizing bacteria. In: E.B. Ward, D.J. Arp, and M.G. Klotz, editors. Nitrification. ASM Press, Washington, DC. p. 39-55. doi:10.1128/9781555817145.ch3

Norton, J.M., and J.M. Stark. 2011. Regulation and measurement of nitrification in terrestrial systems. In: M.G. Klotz, editor. Methods in enzymology. Academic Press, Burlington, VA. p. 343-368.

Qian, Y.L., and M.C. Engelke. 1999. Influence of trinexapac-ethyl on diamond zoyiagrass in a shade environment. Crop Sci. 39:202–208.

Reedlich, L.M., M.D. Millican, and P.L. Koch. 2017. Temperature impacts on soil microbial communities and potential implications for the biodegradation of turfgrass pesticides. J. Environ. Qual. 46:490–497. doi:10.2134/jeq2017.02.0067

Reicher, Z.J., P.H. Dernoeden, and D.S. Richmond. 2013. Insecticides, fungicides, herbicides, and growth regulators used in turfgrass systems. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 891–932.

Robertson, G.P., and P.M. Groffman. 2007. Nitrogen transformations. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 341-364.

Savazzini, F., C.M.O. Longa, and I. Pertot. 2009. Impact of biocontrol agent *Trichoderma atroviride* SC1 on soil microbial communities of a vineyard in northern Italy. Soil Biology and Biochemistry. 41:1457-1465. doi:10.1016/j.soilbio.2009.03.027

Schlesinger, W.H., and J.A. Andrews. 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48:7-20.

Schmidt, M.W.I., Torn, M.S., S. Abiven, T. Dittmar, G. Guggenberger, I.A. Janssens, M. Kleber, I. Kögel-Knabner, J. Lehmann, D.A.C. Manning, P. Nannipieri, D.P. Rasse, S. Weiner, and S.E. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478:49–56. doi:10.1038/nature10386

Shi, W., E. Dell, D. Bowman, and K. Iyyemperumal. 2006. Soil enzyme activities and organic matter composition in a turfgrass chronosequence. Plant Soil 288:285–296. doi:10.1007/s11104-006-9116-1

Singh, A., J.D. Van Hamme, and O.P. Ward. 2007. Surfactants in microbiology and biotechnology: Part 2. Application aspects. Biotechnology Advances 25:99-121.

Sinsabaugh, R.L., M.M. Carreriro, and S. Alvarez. 2002. Enzyme and microbial dnamics of litter decomposition. In: R.G. Burns and R.P. Dick, editors, Enzymes in the environment: Activity, ecology, and applications. Marcel Dekker, New York, NY. p. 567-596.

Steinke, K., and E.H. Ervin. 2013. Turfgrass ecology. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 347–381.

Sterngren, A.E., S. Hallin, and P. Bengtson. 2015. Archaeal ammonia oxidizers dominate in numbers, but bacteria drive gross nitrification in N-amended grassland soil. Front. Microbiol. 6:1-8. doi:10.3389/fmicb.2015.01350

Stier, J.C., J.N. Rogers, III, J.R. Crum, and P.E. Rieke. 1999. Flurprimidol effects on Kentucky bluegrass under reduced irradiance. Crop Sci. 39:1423–1430.

van Bruggen, A.H.C., and A.M. Semenov. 2000. In search of biological indicators for soil health and disease suppression. Appl. Soil Ecol. 15:13–24.

van der Heijden, M.G.A., R.D. Bardgett, and N.M. van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296–310. doi:10.1111/j.1461-0248.2007.01139.x

Veeh, R.H., W.P. Inskeep, and A.K. Camper. 1996. Soil depth and temperature effects on microbial degradation of 2,4-D. J. Environ. Qual. 25:5–12.

Verma, M., S.K. Brar, R.D. Tyagi, R.Y. Surampalli, and J.R. Valéro. 2007. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochemical Engineering Journal. 37:1-20. doi:10.1016/j.bej.2007.05.012

Voroney, R.P. 2007. The soil habitat. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 25-49.

Wessén, E., and S. Hallin. 2011. Abundance of archaeal and bacterial ammonia-oxidizers – Possible bioindicator for soil monitoring. Ecol. Indic. 11:1696-1698. doi:10.1016/j.ecolind.2011.04.018

Wong, P.T.W., and J.H. McBeath. 1999. Plant protection by cold-adapted fungi. In: R. Margesin, and F. Schinner, editors, Biotechnological applications of cold-adapted organisms. Springer, Berlin, Heidelberg. p.177-190.

Wyngaard, N., D.H. Franklin, M.Y. Habteselassie, A. Mundepi, and M.L. Cabrera. 2016. Legacy effect of fertilization and tillage systems on nitrogen mineralization and microbial communities. 80:1262-1271. doi: doi:10.2136/sssaj2016.03.0070

Yao, H., Z. He, M.J. Wilson, and C.D. Campbell. 2000. Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microb. Ecol. 40:223–237. doi:10.1007/s002480000053

Ye, R., A.L. Wright, K. Inglett, Y. Wang, A.V. Ogram, and K.R. Reddy. 2009. Land-use effects on soil nutrient cycling and microbial community dynamics in the Everglades Agricultural Area, Florida. Commun. Soil Sci. Plan. 40:2725–2742.

Zhang, X.Z., E.H. Ervin, and R.E. Schmidt. 2003. Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. Crop Sci. 43:952–956.

CHAPTER 3

EVALUATING THE EFFECTS OF TURF PRODUCTS ON MICROBIAL COMMUNITIES[1]

[1] Diera, A. A., Habteselassie, M.Y., Cabrera, M.L., and Raymer P.L. To be submitted to *Applied Soil Ecology*

ABSTRACT

Resource use efficiency is a primary focus of research in the turf industry in order to reduce costs for turf management programs and promote sustainable turf systems. The microbiology in soils contributes important ecosystem functions that include nutrient cycling and organic matter decomposition that support healthy turf growth. Minimal research examining microbiology in golf courses has been conducted, but interest among researchers and professionals in the turf industry is growing. A 6-mo study at a golf course in Johns Creek, GA was conducted to evaluate the short-term effects of select wetting agents, PGRs, and the microbial inoculant *Trichoderma atroviride* on microbial abundance and function. Trials testing Cascade Plus and Duplex (C+D), Revolution (Rev), and PlantHelper (PH) were initiated in March 2017 in a 4 × 5 randomized complete block (RCB) design in 1.6-m × 2.3-m experimental plots. PrimoMaxx (PM) and Cutless MEC (CL) were applied in separate 1.5-m × 3.0-m experimental plots in May 2017 along with NTC in a 3 × 5 RCB design. Soil samples were collected 4 h, 2 wks, and periodically throughout the growing season after treatment applications. Microbial abundance was evaluated using quantitative polymerase chain reaction (qPCR) assays to quantify total bacteria, total fungi, ammonia-oxidizing bacteria (AOB) and archaea (AOA). Microbial function was evaluated by measuring activities of phosphatase, urease, and soil respiration. Bacteria and AOB groups were sensitive to wetting agents in the putting green, while the only group to exhibit any response to PGR treatments in the fairway was bacteria to PM. Phosphatase activity was unaffected by all treatment applications, but urease activity was stimulated by C+D in the putting green and depressed in CL in the fairway. Soil respiration data suggested treatment applications had immediate, short-term effects on microbial activity in the

putting green and fairway. These results provide preliminary data to help turf managers make better informed decisions about best management practices.

INTRODUCTION

Optimizing resource use efficiency is a critical component in turfgrass maintenance in order to promote long-term, sustainable turf while mitigating associated economic and environmental costs. Central challenges involved in turf management today are epitomized in golf course maintenance. Golf courses, like all turfgrass systems, are inherently anthropogenic and require relatively high intensity management compared to agriculture or grassland systems. Turfgrasses are perennial and require three times more water than corn, the United States' most irrigated crop (Falk, 1976; Milesi et al., 2005). Thus, optimizing management practices is key to maintaining well-irrigated and fertilized golf courses while curtailing the economic and ecological costs to do so. Superintendents are under pressure to cultivate resilient, uniform turfgrass with vigorous rooting capability under a variety of stresses that include longer growing seasons, extreme weather events, soil compaction, and increasing pressure to optimize resource use efficiency and reduce chemical fertilizer and pesticide inputs (Strandberg et al., 2012; Johnson et al., 2013; Breuninger et al., 2013; Stier et al., 2013). Heavy rainfall, drought, heat, thatch accumulation, and disease are among many obstacles to turfgrass productivity (Christians et al., 2016). Superintendents typically use organic and biological products such as wetting agents, plant growth regulators, and microbial inoculants to mitigate the impact of turfgrasses stresses into their maintenance regimes (Christians et al., 2016; Reicher et al., 2013; Nelson and Craft, 1998). The impact of these turf care products on the soil microorganisms driving turfgrass productivity in golf course soils has not been extensively explored.

Golf courses consist of several playing surfaces that each undergoes unique management regimes impacting soil microbial communities specific to the type of land cover (Bartlett et al., 2007). Putting greens and teeing grounds are made up of constructed sand-based root zones with

drainage systems installed to improve nutrient retention, minimize soil compaction, and ensure playable, uniform turf surfaces (Bigelow and Soldat, 2013; Christians et al., 2016). These surfaces receive the highest application frequency of fertilizers, wetting agents, and other turf care products compared to the fairways and roughs (Bartlett et al., 2007; 2009). Although fairways are also highly managed systems, these spaces are designed to accommodate continual foot traffic and playability. Native soil conditions typically inform management approaches, but a topdressing, (a mix of sand and peat moss), is often incorporated into finer soil textures to increase porosity and promote water filtration. Peat moss alone or other organic substrates may be added to coarser soils to improve nutrient retention (Bigelow and Soldat, 2013).

Management intensity negatively correlates to microbial community size among different golf course playing surfaces, and sand content affects the phenotypic expression of microbial community structure due to pore space and resource access (Bartlett et al., 2009). However, microbial communities in all golf course soils are quick to establish themselves and are similar to one another compared to other microbial communities in other land use types (Bartlett et al., 2009; 2007).

Fostering robust microbial communities that drive turfgrass productivity is essential for promoting long-term soil health. According to Doran and Zeiss (2000), soil health is "the capacity of soil to function as a vital living system to sustain biological productivity, promote environmental quality, and maintain plant and animal health." The rhizosphere provides the primary media for biological activity in turfgrass soils, including turfgrass development and microbial activity (Bigelow and Soldat, 2013). Organic matter decomposition, nutrient cycling, and turfgrass resilience involve processes driven by microbial action that directly impact soil health (Doran and Zeiss, 2000; Barrios, 2007; Gaussoin et al., 2013; Morgan et al., 2005; van der

Heijden et al., 2008). In turfgrass, decomposition mediates the build-up of thatch, a compact layer formed between the turf canopy and soil surface as a result of intermingling root tissues and decomposing organic residues, which can otherwise limit root growth, limit air and water flow, and increase the vulnerability of turfgrass to disease (Gaussoin et al., 2013; Christians et al., 2016). Furthermore, microbial decomposers are responsible for organic matter breakdown into plant-available nutrients like carbon (C), nitrogen (N), phosphorus (P), and others essential to turfgrasses (Schmidt et al., 2013; Gaussoin et al., 2013; Kandeler, 2007; van der Heijden et al., 2008; Barrios, 2007). Healthy soils support the proliferation of microorganisms that provide beneficial services to turfgrass such as enhanced nutrient acquisition, antibiotic protection against pathogens, and improving plant survivability under abiotic stresses from the environment such as heat, drought, and soil compaction (Powell and Klironomos, 2007; Barrios, 2007; Christians et al., 2016; Malinowski et al., 2000; Gaussoin et al., 2013).

Resilient, stable responses to stress and disturbance are central to a healthy soil ecosystem (Doran and Zeiss, 2000). Parameters such as microbial abundance, enzyme activity, and soil respiration can serve as simple indicators of soil health. Microbial abundance and diversity are practical soil quality indicators because microbial communities are influenced by different kinds of land use and vegetation (Doran and Zeiss, 2000; Yao et al., 2000). Functional redundancy is expected from many soil microbial species, thus quantifying broader microbial groups such as bacteria, fungi, and ammonia-oxidizers is an effective approach to evaluating biodiversity (Barrios, 2007; van Bruggen and Semenov, 2000). Bacteria and fungi are copiotrophs and oligotrophs respectively, and the predominance of one group over the other is indicative of nutrient availability, habitat succession, and soil physiochemical properties such as pH (Heijden et al., 2008; Fierer and Jackson, 2007; Kuramae et al., 2010). Ammonia-oxidizing

archaea (AOA) and bacteria (AOB) are microbial groups involved in autotrophic nitrification, the first step in ammonia (NH_3) reduction, in a soil system (Hatzenpichler, 2012; Norton and Stark, 2011; Norton, 2011). Measuring enzyme activity elucidates microbial processes including those involved in nutrient cycling (Barrios, 2007; Kandeler, 2007; Shi et al., 2006). Many microbial extracellular enzymes stabilize and remain active after microorganisms have decayed (Burns et al., 2013). Phosphatase and urease are two such enzymes that are microbially-secreted as a means to mineralize organically-bound phosphorus (P) and urea-bound NH_3 (Plante, 2007; Mobley and Hausinger, 1989; Kandeler, 2007). The stability of soil organic matter (SOM), the largest terrestrial source of carbon dioxide (CO_2) consisting of humic substances, plant, animal, and microbial biomass at every stage of life and decay is dependent on microbial activity (Schlesinger and Andrews, 2000; Kandeler, 2007; Schmidt et al., 2011; Kuzyakov et al., 2000). Rates of soil respiration can estimate SOM decomposition based on CO_2 evolution and discern energy flow within a soil food web system that drives nutrient mineralization, soil stabilization, and improved water permeability (Kandeler, 2007; Barrios, 2007).

Golf course management programs aim to maintain turfgrass resiliency by incorporating a variety of products to relieve abiotic and biotic stresses on turf. Such products currently sold on the market today include wetting agents, plant growth regulators (PGRs), and microbial inoculants. Wetting agents are alcohol-based, amphipathic surfactants applied to relieve localized dry spots (LDS) caused by soil hydrophobicity common in sand-based golf courses (Kostka 2000; Barton and Colmer, 2011). PGRs target growth hormones in turfgrass that promote root growth and inhibit shoot development to improve turf density, uniformity, and resilience to heat, drought, shade, traffic, mowing, and other stresses (Stier et al., 1999; Qian and Engelke, 1999; Reicher et al., 2013; Lickfeldt et al., 2001; Zhang et al., 2003). Microbial

inoculants have a variety of applications including suppression of common turfgrass diseases such as *Sclerotinia homoecarpa*, *Rhizoctonia solani*, and *Pythium* spp. (Nelson and Craft, 1998; Martinez et al., 2017). *Trichoderma atroviride* is a beneficial fungus that provides turfgrass antifungal resistance to such pathogenic fungi (Wong and McBeath, 1999; Verma et al., 2007; Harman et al., 2004; 2006; Daryaei et al., 2016a; Daryaei et al., 2016b).

Wetting agents, PGRs, and *Trichoderma* spp. products are commonly incorporated into golf course management programs, but their impact on beneficial microorganisms driving turfgrass productivity has not been extensively researched. Further scientific data is needed to evaluate the effects of such products on soil microbial communities and their ecosystem services as interest in soil microbiology continues to expand in the turf industry. This study was designed to address the short-term dynamics between turf care products and soil microbial communities in golf course soils. The primary objective was to evaluate the short-term impacts of select wetting agents, PGRs, and *T. atroviride* on the abundance and activities of microbial communities in systems. Previous research indicates that shifts in microbial communities can be expected due to seasonal changes and other environmental factors beyond the parameters of this study (Savazzini et al., 2009; Dell et al., 2008; Mueller and Kussow, 2005; Beirn et al., 2017; Voroney, 2007). Short-term responses to treatments are anticipated, but microbial abundance and activity are expected to stabilize over time (Bartlett et al., 2007).

MATERIALS AND METHODS

Study sites and sample collection

Two field trials were established at Rivermont Country Club in Johns Creek, GA in spring 2017 using randomized complete block designs to observe the effects of five turf care products on microbial community abundance, composition and activities in putting green and

fairway soils. Wetting agents Cascade Plus combined with Duplex (Precision Laboratories, Waukegan, IL) and Revolution (Aquatrols, Paulsboro, NJ) were tested in the putting green soil along with Plant Helper (AmPac Biotech, Fresno, CA) between March 14 and June 6, 2017 (Table 3.1). Plant growth regulator (PGR) products PrimoMaxx (Syngenta, Greensboro, NC) and Cutless MEC (SePRO, Carmel, IN) were tested in the fairway soil between May 16 to August 22, 2017 (Table 3.2). Total rainfall over the course of the putting green and fairway trials were 32 cm and 59 cm respectively; average temperature ranged from 0.8ºC to 26ºC throughout the putting green trial and 18ºC to 29ºC in the fairway trial (AEMN, 2018). In each trial, two sensors and a CR1000 datalogger (Campbell Scientific, Logan, UT) were installed to capture soil temperature and moisture data beginning May 14, 2017 through July 13, 2017. Average soil temperature ranged from 22ºC to 29ºC in the putting green and 21ºC to 29ºC in the fairway trial; average soil moisture ranged from 26% to 36% in the putting green and 6.8% to 11% in the fairway.

Putting green trial

Cascade Plus and Duplex (C+D), Revolution (Rev), and PlantHelper (PH) were applied to 2.59-m × 1.52-m experimental plots on a putting green sandy soil with 3% soil organic matter (SOM) and an average pH of 6.5 in 0.01 M $CaCl_2$. All treatments and non-treated control (NTC) plots were replicated five times. Treatments were measured using half labeled rates in each tank mix to provide double coverage over each plot. Experimental plot dimensions were used to calculate the total surface area needed to provide double-coverage for five replications of one treatment: 39.5 m^2. Calibrations were determined by filling a 15-L backpack sprayer with 7.6 L of water and covering the total surface area of 39.5 m^2 per treatment. The difference between the 7.6 L and unused water was 2.54 L, and equation (1) was used to prepare each tank mix to cover

92.9 m^2, the metric expression of an area size commonly used in application rates of turf care products.

$$\text{Water in tank mix (L)} = \left(\frac{92.9\ m^2 \times 2.54\ L}{39.5\ m^2}\right) - x\ \text{mL treatment} \quad (1)$$

The water volume did not have to be altered for the tank mix of PlantHelper (PH) treatments, because the product is a dry powder. Applications were repeated 31, 64, and 92 days after treatment (DAT).

Composite soil samples of 5 to 7 soil cores were collected 4 hours after treatment (0 DAT), 14, and 84 DAT using a 127-mm soil prove to collect 10-cm soil columns. A sterile knife was used to separate the top and lower 5 cm of each soil column in individual Ziploc bags. Soil samples were stored at 4ºC until processed through a 0.2-µm sieve to remove plant debris and used for standard culture methods, enzyme assays, and soil respiration analysis. Approximately 3 g of each soil sample were separated in Ziploc bags and stored at -20ºC for quantitative polymerase chain reaction (qPCR) analysis.

Fairway trial

PrimoMaxx (PM) and Cutless MEC (CL) were applied to 3.0-m × 1.5-m experimental plots on a sandy clay loam fairway soil with an average pH of 6.1 in 0.01 M $CaCl_2$ (Table 3.2). Each treatment and NTC plot was replicated five times and prepared as described in the putting green trial at half field use rates to provide double coverage with a backpack sprayer. Seventy-four percent of the labeled use rate of Cutless MEC (CL) was applied 0 DAT. All subsequent treatment applications were repeated 29 and 59 DAT at labeled field rates.

Composite soil samples of 5 to 7 soil cores were collected 4 hours after treatment (0 DAT), 15, 42, 63, and 98 DAT using a drill marked at 5- and 10-cm and a plastic bucket. The top 5-cm soil samples were collected in each plot prior to collecting samples from the soil depth

of 5 to 10 cm. Soil samples were stored in separate Ziploc bags at 4ºC. Due to the high clay and soil moisture content, samples were processed manually to remove plant debris and used for standard culture methods, enzyme assays, and soil respiration analysis. Approximately 3 g of each soil sample were separated in Ziploc bags and stored at -20ºC for qPCR analysis.

Soil sample and data analysis

Soil weight

Dry soil weight was determined for all soil samples by analyzing moisture content. Wet soil weights ranging from 1 to 2 g of each sample were recorded. Samples were placed in a drying oven at 100ºC for 24 h and cooled to room temperature in a desiccator for 2 h. Dry sample weights were recorded, and equation (2) was used to calculate dry soil weight (g) for each sample:

$$\text{Dry soil weight (g)} = 1 - \left(\frac{\text{Wet soil wt (g)} - \text{Dry soil wt (g)}}{\text{Dry soil wt (g)}}\right) \quad (2)$$

Quantitative polymerase chain reaction

Quantitative polymerase chain reaction (qPCR) assays were used to estimate total bacteria, total fungi, AOA, and AOB populations in soil samples collected from the upper 5 cm soil depth in both trials. Soil DNA was extracted from 0.25 g of each soil sample using the DNeasy PowerSoil DNA extraction kit (QIAGEN, Germantown, MD). Table 3.3 describes the target genes, amplicon lengths, primers, and thermal cycling conditions used to quantify total bacteria, total fungi, AOA, and AOB respectively. The reaction volume for qPCR was 20 µL that contained 2× PowerUp SYBR Green Master Mix (ThermoFisher Scientific), 2 µL of DNA template, 0.8 µL of forward primers, 0.8 µL of reverse primers, and 6.4 µL of nuclease-free PCR water. Serial dilutions of stock target organisms ranging from 30 to 3×10^5 copies of DNA per µL were prepared and run in analytical triplicate for total bacteria, total fungi, and AOB assays.

StepOne Software (Applied Biosystems) was used to analyze generated qPCR data. The qPCR reaction efficiencies and R^2 values for standard curves generated by StepOne ranged from 72% to 124% and 0.968 to 0.999 respectively. The standard curves and equation (3) were used to calculate the quantity of DNA (copy g^{-1}) in each soil sample.

$$\text{copy } g^{-1} = \frac{x \text{ copies}}{2\ \mu L} \times \left(\frac{100\ \mu L}{0.25\ g}\right) \quad (3)$$

Phosphatase assay

Soil samples collected from 0 to 5 cm and 5 to 10 cm below the soil surface were colorimetrically analyzed to estimate the rate of phosphatase activity (µmol phosphate evolved─$g^{-1} \cdot h^{-1}$) as an indicator of soil P cycling (Tabatabai, 1994). Two 16-mL glass scintillation vials were obtained for each soil sample and wrapped in aluminum foil to minimize light exposure. One gram of soil and 4 mL of Tris-maleate buffer (pH 7.0) were added to each vial. One milliliter of 100-mM para-nitrophenyl phosphate (pNPP) was added to one of two vials per sample, while the other vial was used as a control. Vials were shaken for 30 min on a rotary shaker at 175 rpm. One milliliter of pNPP was added to each control vial, and 1 mL of 0.5-$CaCl_2$ and 4 mL of 0.5-M NaOH were added to all vials to terminate activity. Contents of each vial were transferred to 16-mL polystyrene centrifuge tubes and centrifuged for 10 min at 10,000 rpm at 4ºC. The absorbance of the supernatant from each vial was colorimetrically analyzed using a spectrophotometer at 400 nm. Select samples were diluted to 1:25 when absorbance was too high to detect. Standard curves were derived by preparing standard solutions ranging from 0 to 10 µM *p*-nitrophenol in Tris-maleate buffer for each set of samples by collection date. Linear equations derived from standard curves ($R^2 \geq 0.9905$) were used to calculate phosphate concentrations in each vial (µmol phosphate L^{-1}), because one mole of phosphate is produced by one mole of *p*-nitrophenol. The difference between phosphate

concentrations in the treated and control vials were used in equation (4) to determine phosphatase activity (μmol phosphate evolved─$g^{-1} \cdot h^{-1}$) in each soil sample:

$$\mu\text{mol } P_i \text{ evolved─g}^{-1} \cdot \text{h}^{-1} = \frac{\mu\text{mol } P_i}{\text{L}} \times \frac{10 \text{ mL}}{1 \text{ g}} \times \frac{1 \text{ L}}{1000 \text{ mL}} \times 2 \times \text{dilution factor} \quad (4)$$

Urease assay

Soil samples collected from 0 to 5 cm and 5 to 10 cm from the soil surface were analyzed using a 2% boric-acid trap method to estimate the rate of urease activity (μmol NH_3 evolved─$g^{-1} \cdot h^{-1}$) as an indicator of soil N cycling (Mobley and Hausinger, 1989). For each sample, two biplate petri dishes were prepared by adding 1 g of soil and 3 mL of 0.5-M Tris-maleate buffer solution (pH 7.0) with 1% sodium azide in one compartment of each petri dish. Three milliliters of 2% boric acid indicator solution were pipetted into the second compartment of each petri dish. To initiate enzyme activity, 1 mL of 6-M urea was added to the soil and buffer solution in one replicate of each soil sample. The same compartment of the second replicate of each sample received 1 mL of distilled water to account for the release of ammonia from the background ammonium (NH_4^+). Petri dishes were allowed to incubate for 1 h at room temperature. One-half milliliter of 10-mM $AgSO_4$ solution and 3-M K_2CO_3 solution were added to terminate urease activity and release evolved NH_3 into the boric acid trap. Plates were secured in Ziploc bags and continued to incubate at room temperature for 24 h. Boric acid solutions were titrated using 0.02-*N* HCl solution. The rate of urease activity for each soil sample was calculated by applying equation (5) to the amount of HCl used to titrate each petri dish and subtracting the value of the control from the value of the treated plate.

$$\mu\text{mol } NH_3 \text{ evolved─g}^{-1} \cdot \text{h}^{-1} = \frac{\text{mL HCl}}{\text{adjusted soil weight (g)}} \times \frac{0.02 \text{ mol}}{\text{L}} \times \frac{1 \text{ L}}{1000 \text{ mL}} \times \frac{10^6 \text{ μmol}}{\text{mol}} \quad (5)$$

Soil respiration

Three of five replicates from each treatment were selected to measure soil respiration (mg CO_2 evolved 1 g^{-1}) and examine effects of treatments on overall microbial activity. Five grams of soil from each soil depth were mixed in separate mason jars for each sample totaling 10 g soil per jar. An empty mason jar was obtained for each treatment group of soil samples to serve as a control to capture background CO_2. Glass beakers containing 10 mL of 0.08-*N* $Ba(OH)_2$ were placed in each mason jar to capture evolved CO_2. Sealed jars were groups in Ziploc bags based on treatments and allowed to incubate in the dark at 22ºC for 24 h. With phenolphthalein as indicator, 0.08-*N* HCl was used to titrate the $Ba(OH)_2$ traps. The CO_2 from control jar was subtracted from the jars with soil. Soil respiration rate (mg CO_2 evolved g^{-1}) was estimated with equation (6) where *x* was the volume of HCl that was used to titrate the trap.

$$\text{mg } CO_2 \text{ g}^{-1} = [(0.08\ N\ Ba(OH)_2 \times x \text{ mL } Ba(OH)_2) - (0.08\ N \text{ HCl} \times x \text{ mL HCl})] \times \frac{22 \text{ mg } CO_2}{10 \text{ g} * \text{adjusted soil weight (g)}} \quad (6)$$

Statistical analysis

Each response variable in the qPCR, phosphatase, and urease assays were averaged by each treatment per sampling day and soil depth separately in both trials. Data were analyzed using mixed-model analysis in JMP Pro 13. Treatment and soil depth were categorical variables, sampling day was a continuous variable, and experiment plot was treated as random effect in the mixed models. Soil depth was excluded from the models run for microbial abundance. Normality and equal variance assumptions of the residuals from each mixed model were checked for violations. Datasets of total fungi and urease activity in the putting green trials underwent a square root transformation, because each corresponding model violated assumptions of normality and equal variance respectively. Wald tests were conducted to test the significance of experimental plot location as an explanatory variable in each model. Because mixed-models

were ill-fitting as demonstrated by negative R^2 values, total bacteria and AOB population data in the fairway trial were separated by sampling day and analyzed using one-way analysis of variance (ANOVA) comparing average population size versus treatment. One-way ANOVA models were also used to examine significant differences among rates of urease activity in the putting green and fairway trials. Tukey's honest significant difference (HSD) test was used to conduct post hoc analyses to identify significant relationships among treatments within all models.

Soil respiration averages in each treatment were separated by sampling day and analyzed using one-way ANOVA comparing treatment to population size, because mixed models were ill-fitting to describe the data. Tukey's HSD was initially used to conduct post hoc analyses of significant models but was unable to detect nonlinear mean comparisons 0 DAT in the putting green trial. Least significant differences (LSD) among all possible mean comparisons were conducted using Student t-tests.

RESULTS AND DISCUSSION

Microbial abundance

Bacteria dominated the microbial communities in the top 5 cm of the putting green and fairway soils with mean population sizes of 5.1×10^7 copies g^{-1} and 3.2×10^7 copies g^{-1} respectively (Table 3.4). The distribution of populations in both trials was total bacteria > total fungi > AOB > AOA across all treatments and sampling days. The predominance of bacteria over fungi in both soils was not surprising given that bacteria are more abundant on earth, and both soils are mildly acidic, highly disturbed, and receive frequent fertilizer applications. Greater biological activity is associated with bacteria-dominated systems compared to those driven by fungi, because nutrient availability is high (van der Heijden, 2008; Fierer et al., 2007).

The similarity in community structures between the putting green and fairway agree with previous findings observing comparable microbial communities among United States Golf Course (USGA) soils predominated by Gram-negative and methanotrophic bacteria (Bigelow et al., 2002; Bartlett et al. 2007). The ratio of AOB to AOA population means was nearly three-fold in the fairway (74:1) than the putting green (27:1). Ammonia availability drives AOB activity, and the cooler late spring to early summer trial period of the putting green trial likely attributed to the relatively lower AOB abundance than that observed in the fairway (Wyngaard et al., 2016; Ouyang et al., 2016; Habteselassie et al., 2013).

Bacteria and AOB populations, averaged from all samples collected from the duration of the trial, exhibited significant responses to wetting agents (C+D and Rev) and PH treatments in the putting green (Table 3.5). Total bacteria and AOB population means in all treatments did not recover to comparable population means in the NTC samples (Table 3.6). Average abundance of total bacteria was twice as high in the NTC samples than those from the C+D, Rev, and PH plots, while mean AOB abundance was an order of magnitude lower in all treated samples compared to the NTC. Mean AOA and total fungi populations were unaffected by C+D, Rev, and PH treatments (Table 3.5). The response to wetting agents (C+D and Rev) and *T. atroviride* inoculant (PH) suggests possible bacterial sensitivity to the products.

In the fairway, the abundance of total fungi (4.1×10^4 copy g^{-1} to 5.1×10^4 copy g^{-1}), AOA (6.6×10^3 copy g^{-1} to 7.6×10^3 copy g^{-1}), and AOB (4.9×10^5 copy g^{-1} to 5.1×10^5) were not significantly altered by PM and CL treatment applications (Tables 3.7 and 3.8). Total bacteria population means were also comparable when pooled by treatment except 42 DAT in which PM samples had higher abundance than NTC samples (Table 3.9). Increased root development from two monthly applications of PM may have influenced this spike in bacterial proliferation. The

recovery towards comparable AOB abundance means among treatments in subsequent sampling days may be attributed to mature turfgrass residue later in the growing season. However, the overall lack of PM effect on microbial communities is consistent with previous research (Feng et al., 2002).

Total fungi, AOA, and AOB abundances among all treatments significantly changed over time in the putting green (Table 3.5). From late spring to early summer, average total fungi populations declined throughout the duration of the experiment (Figure 3.1). As the early growing season progressed, the increasingly copiotrophic conditions from biological activity and warming temperatures may have influenced the decline in fungi abundance, because fungi tend to prevail in harsher, nutrient-poor environments (van der Heijden, 2008; Fierer et al., 2007). AOA abundance means increased between 0 DAT and 14 DAT and stabilized between 14 DAT and 84 DAT (Figure 3.2). Mean AOB abundance stabilized in the C+D and PH treatments and declined in the Rev and NTC over the duration of the experiment (Figure 3.3). The growth patterns of mean AOA and AOB abundances in the putting green suggest both populations stabilized early in the growing season. No clear pattern was detected among microbial abundance, soil moisture, and rainfall data, although consistently high soil moisture levels were indicative of regular irrigation in the putting green. The microbial community in this soil may be well-suited to high soil moisture conditions, although further research is recommended.

In the fairway, AOA abundance gradually increased in the PM (7.8×10^3 copy g^{-1} to 9.0×10^3 copy g^{-1}) and CL treatments (5.8×10^3 copy g^{-1} to 8.7×10^3 copy g^{-1}) and decreased in the NTC (1.2×10^4 copy g^{-1} to 6.5×10^3 copy g^{-1}) from May to August (Figure 3.4). Mean AOB abundance significantly increased from the beginning of the fairway trial to the final sampling day (Figure 3.5). Increased SOM and/or NH_3 availability from the ongoing growing season

likely attributed to higher AOB abundance in the fairway (Dell et al., 2008). The fluctuating soil moisture levels cycling between dry and saturated conditions in response to a rainy growing season also may have attributed to the overall increase in AOB populations and decrease in AOA abundance over the growing season. AOB readily mobilize and proliferate in the presence of water and become competitive against AOA (Hatzenpichler, 2012). Microbial responses to seasonal changes are well-documented, although recent research has also observed many other influential factors on microbial abundance in turfgrass soils specific to location such as management, soil type, plant cover, and P availability (Beirn et al., 2017; Elliott et al., 2008; Bartlett et al., 2009; Bigelow et al., 2002; Kuramae et al., 2010). The fairway soil experienced fluctuating soil moisture levels ranging from dry to saturated conditions in response to a rainy growing season.

The location of experimental plots did not have a significant influence on any mixed models describing microbial abundance data in putting green or fairway trials.

Phosphatase activity

Phosphatase activity ranged from 0.11 P_i—g^{-1},h^{-1} to 198 P_i—g^{-1},h^{-1} in the putting green and 35 P_i—g^{-1},h^{-1} to 1,059 P_i—g^{-1},h^{-1} in the fairway (Table 3.4). Treatment applications did not influence mean rates of phosphatase activity in either trial (Table 3.10). Furthermore, the location of experimental plots did not have a significant effect on any mixed models describing phosphatase data in each trial. However, phosphatase activity was significantly influenced by sampling date, soil depth, and the interaction term between time and depth in both trials.

In the putting green, phosphatase activity increased throughout the duration of the trial at both soil depths. Mean phosphatase activity in the soil samples collected from the top 5 cm nearly doubled from 66 µmol P_i—g^{-1},h^{-1} at the beginning of the trial to 119 µmol P_i—g^{-1},h^{-1} 84

DAT. Samples collected from 5 to 10-cm soil depth exhibited a modest increase in mean phosphatase activity from 17 µmol P_i—$g^{-1,}h^{-1}$ 0 DAT to 30 µmol P_i—$g^{-1,}h^{-1}$ 84 DAT. The availability of P from fertilizers in the putting green paired with increased microbial activity associated with warming temperatures likely attributed to the overall rise in phosphatase activity. In the fairway, phosphatase activity decreased over time at each soil depth. Average phosphatase activity in the soil samples collected from the top 5 cm declined from 705 µmol P_i—$g^{-1,}h^{-1}$ (0 DAT) to 251 µmol P_i—$g^{-1,}h^{-1}$ (98 DAT). In soil samples collected from 5 to 10-cm depth, mean phosphatase activity decreased from 498 µmol P_i—$g^{-1,}h^{-1}$ to 300 µmol P_i—$g^{-1,}h^{-1}$ 0 DAT and 98 DAT respectively. The higher rates of phosphatase activity in the fairway compared to the putting green is associated with less disturbance, higher levels of organic material, and a higher clay fraction (24% clay) in the fairway. Soil moisture and rainfall did not appear to correlate with phosphatase activity in either trial.

The higher mean rates of phosphatase activity observed in the top 5 cm of the rhizosphere of both soils agree with previous work by Wright and Reddy (2001) who concluded phosphatase activity negatively correlates to soil P and microbial biomass C. High biological activity in the thatch-mat later is promoted by living turfgrass and decaying organic material accumulating at the soil surface (Christians et al., 2016). Organically-bound P and inorganic P fertilizers are easily accessible to microorganisms at the soil surface and become less available at increasing soil depths (Holden and Fierer, 2005; Tate 1979; Duxbury and Tate, 1981).

Urease activity

Urease activity ranged from 0 µmol NH_3—$g^{-1,}h^{-1}$ to 6.2 NH_3—$g^{-1,}h^{-1}$ in the putting green and 8.4 µmol NH_3—$g^{-1,}h^{-1}$ to 130 NH_3—$g^{-1,}h^{-1}$ in the fairway (Table 3.4). Treatment

applications significantly influenced urease activity in the putting green and fairway soils (Table 3.10).

In the putting green, mean urease activity pooled by treatment was significantly higher in the C+D samples (2.3 NH_3—g^{-1},h^{-1}) compared to mean urease activity of 1.3 NH_3—g^{-1},h^{-1} in the NTC, Rev, and PH samples. The random effect of experimental plot location was significant in the putting green which indicates site-specific variability among the trial data. The three-way interaction term among treatment, soil depth, and sampling date effects identified comparable mean rates of urease activities among treatments at the initiation of the trial in each soil depth (Table 3.11). The interaction between treatment and soil depth effects reiterated higher mean urease activity occurred in the C+D treatment 84 DAT than the NTC, Rev, and PH treatments. Urease activity declined approximately 40% between 0 DAT and 14 DAT in rhizosphere and slowly recovered over time in the NTC, Rev, and PH treatments. Mean rates of urease activity among these treatments were consistently low throughout the duration of the trial in the 5 to 10-cm soil depth. The initial drop in urease activity during the first two weeks of the trial and overall decline in these treatments signified that Rev and PH treatments did not affect urease activity. The significant increases in urease activities throughout the duration of the trial at both soil depths (0.93 NH_3—g^{-1},h^{-1} in the top 5 cm; 3.0 NH_3—g^{-1},h^{-1} in 5 to 10-cm depth) suggest C+D stimulated ureolytic microbial activity in the putting green. Urea is hydrolyzed by water and urease enzymes (Killham and Prosser, 2007). The removal of hydrophobic organic material by C+D and increased soil permeability may have released urease enzymes complexed to the soil colloids and increased water flow throughout the soil matrix (Kostka, 2000; Burns et al., 2013). One or both of these phenomena likely explain the treatment effect on urease activity.

In the fairway, average urease activity pooled by treatment was significantly lower in the CL samples (30 NH_3—$g^{-1,}h^{-1}$) than urease activity means of the NTC (37 NH_3—$g^{-1,}h^{-1}$) and PM (34 NH_3—$g^{-1,}h^{-1}$) samples (Table 3.12). The random effect of experimental plot location was not significant in the mixed model. Several interacting causes may be responsible for the decrease in urease activity among the CL treatment. Turfgrass exhibited bronzing within two weeks after initial CL applications, a symptom of air restriction in the root zone. Oxygen stress is also associated with decline in N availability (Smucker and Saettler, 1977). Urease activity did not recover to comparable levels to those in the NTC and PM plots over the course of the trial.

The effects of soil depth on urease activity in the putting green and fairway were similar to those on phosphatase activity. Mean rates of urease activity in putting green pooled by soil depth ranged from 0 NH_3—$g^{-1,}h^{-1}$ to 6.2 NH_3—$g^{-1,}h^{-1}$ in the top 5 cm, and 0 NH_3—$g^{-1,}h^{-1}$ to 4.4 NH_3—$g^{-1,}h^{-1}$ 5 to 10 cm from the soil surface. In the fairway, the significant interaction between soil depth and sampling date identified average urease activity in the top 5 cm was 11 NH_3—$g^{-1,}h^{-1}$ lower in the CL treatment than the NTC while mean urease activity was comparable 5 to 10 cm below the soil surface (Table 3.12). Enzyme activity diffuses at deeper soil depths due to decreasing substrate availability (Holden and Fierer, 2005; Tate 1979; Duxbury and Tate, 1981). Furthermore, increasing enzyme activities in the top 5 cm of the soil were also affected by fluctuating, warmer ambient temperatures from the exposed soil surface compared to the steady, cooler temperatures below the soil surface.

Overall, time did not have a significant effect in the putting green. However, the significant effect of sampling date in the fairway indicated overall urease activity initially spiked from 0 DAT (30 µmol NH_3—$g^{-1,}h^{-1}$) to 14 DAT (47 NH_3—$g^{-1,}h^{-1}$) and declined to 26 µmol NH_3—$g^{-1,}h^{-1}$ by the end of the 98-d trial. These observations in the fairway may be attributed to

a combination of fertilizer applications and increasing plant-microbe competition for N resources as biological activity increases throughout the growing season (van der Heijden et al., 2008; Mueller and Kussow, 2005).

Soil respiration

Soil respiration ranged from 0.06 mg CO_2 g^{-1} to and 0.35 mg CO_2 g^{-1} in the putting green and 0 mg CO_2 g^{-1} to 1.8 mg CO_2 g^{-1} in the fairway (Table 3.4). In the putting green one-way ANOVA models, average soil respiration in the Rev and PH samples were significantly higher than the NTC and C+D samples, but no significant treatment effects on soil respiration 14 DAT and 84 DAT were detected (Table 3.13). The spike in soil respiration 14 DAT in the Rev and PH treatments followed by comparable respiration responses in these soils to the NTC and C+D treatments indicated microbial activity exhibited immediate responses to Rev and PH applications. Microbial activity stabilized to levels similar to those at the beginning of the trial, which signifies native microbial communities were able to recover to pre-treatment levels within 84 d of treatment applications, although a rainfall event 2 d prior to sampling may also have contribute to the relatively lower soil respiration observed at the end of trial.

Mean soil respiration in the fairway fluctuated in PM, CL, and NTC plots at least 42 DAT (Table 3.14). Mean soil respiration was 0.37 mg CO_2 g^{-1} lower in the PM samples compared to the NTC and CL samples 0 DAT. In the CL samples 15 DAT, mean soil respiration was 1.48 mg CO_2 g^{-1} lower than NTC and PM plots. Mean soil respiration in both PM and CL treatments were 0.10 mg CO_2 g^{-1} and 0.07 mg CO_2 g^{-1} higher than mean soil respiration in NTC samples respectively. All three treatments stabilized to comparable levels among treatments through 63 and 98 DAT. The fluctuation in microbial activity throughout the first three sampling days indicate that the soil microbial community in the fairway was initially responsive to

treatment applications but were able to recover from the treatment effects later. Soil respiration responses did not follow any consistent patterns among treatment applications, nor did soil temperature, soil moisture, rainfall, and ambient temperature data elucidate any clear relationship to soil respiration. Therefore, such responses indicate that environmental factors beyond the scope of this study might have influenced soil respiration within the first 6 to 8 wks.

In future research evaluating the soil health of golf courses, the long-term effects of turf care products on biological indicators must be considered (van Bruggen and Semenov, 2000). This work examining the dynamics between turf care products and soil microbial communities in golf course soils is limited by time (<1-yr study) and space (1 site location per treatment), but it provides a framework to expand future research. Wetting agents, PGRs, and microbial inoculants examined in this study and a myriad of others could be applied to putting greens, fairways, teeing grounds, and roughs to improve comparisons of microbial responses in each of these soils (Bartlett et al., 2007; 2009). Long-term responses over several years would elucidate the sustainability of the use of these products in golf course management programs as it relates to the microbiology of these soils. An emphasis on collecting turf quality data is also essential to evaluating the sustainability of golf course programs in future research.

Enzyme assays captured the potential of enzyme activity in a soil, but this information did not reflect active nutrient cycling dynamics due to the inactivity of soil colloid-complexed enzymes (Nannipieri et al., 2002; Burns et al., 2013).

CONCLUSIONS

Short-term responses to turf care products varied among microbial groups, enzyme activities, and soil respiration. Bacteria and AOB groups were particularly sensitive to wetting agents in the putting green, while total bacteria were the only evaluated microbial group to

exhibit any response to PGR treatments, specifically PM, in the fairway. Phosphatase activity was unaffected by all treatment applications, but urease activity was stimulated by the wetting agent treatment C+D in the putting green and depressed in the PGR treatment of CL in the fairway. Soil respiration data suggested immediate, short-term microbial response to treatment applications in the putting green and fairway, although more research is needed to explore this trend further. This study presents some insight into the dynamics between turf care products and soil microbial communities in golf course soils. The practical importance of the study is the provision of information for golf course superintendents and other turfgrass managers to make management decisions that improve the long-term sustainability of their turf systems.

REFERENCES

AEMN. 2018. University of Georgia Weather Network. College of Agricultural and Environmental Sciences. University of Georgia. http://georgiaweather.net (accessed 17 Jul. 2018).

Barrios, E. 2007. Soil biota, ecosystem services and land productivity. Ecol. Econ. 64:269–285. doi:10.1016/j.ecolecon.2007.03.004

Bartlett, M.D., I.T. James, J.A. Harris, and K. Ritz. 2007. Interactions between microbial community structure and the soil environment found on golf courses. Soil Biol. Biochem. 39:1533–1541. doi:10.1016/j.soilbio.2007.01.003

Bartlett, M.D., I.T. James, J.A. Harris, and K. Ritz. 2009. Microbiological profiles of golf course soils. Int. Turfgrass Soc. Res. J. 11:949–956.

Barton, L., and T.D. Colmer. 2011. Ameliorating water repellency under turfgrass of contrasting soil organic matter content: Effect of wetting agent formulation and application frequency. Agric. Water Manag. 99:1–7. doi:10.1016/j.agwat.2011.08.009

Beirn, L.A., J.W. Hempfling, C.J. Schmid, J.A. Murphy, B.B. Clarke, and J.A. Crouch. 2017. Differences among soil-inhabiting microbial communities in *Poa annua* turf throughout the growing season. Crop Sci. 57:S-262–S-273. doi:10.2135/cropsci2016.06.0463

Bigelow, C.A., and D.J. Soldat. 2013. Turfgrass root zones: Management, construction methods, amendment characterization, and use. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 383–423.

Bonkowski, M., C. Villenave, and B. Griffiths. 2009. Rhizosphere fauna: The functional and structural diversity of intimate interactions of soil fauna with plant roots. Plant Soil. 321:213–233.

Borneman, J., and R. J. Hartin. 2000. PCR primers that amplify fungal rRNA genes from environmental samples. Appl. Environ. Microbiol. 66:4356-4360.

Breuninger, J.M., M.S. Welterlen, B.J. Augustin, V. Cline, and K. Morris. 2013. The turfgrass industry. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 37–103.

Burns, R.G., J.L. DeForest, J. Marxsen, R.L. Sinsabaugh, M.E. Stromberger, M.D. Wallenstein, M.N. Weintraub, and A. Zoppini. 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol. Biochem. 58:216-234. doi:10.1016/j.soilbio.2012.11.009

Christians, N.E., A.J. Patton, and Q.D. Law. 2017. Fundamentals of turfgrass management. 5th ed. John Wiley & Sons, Hoboken, NJ.

Daryaei, A., E.E. Jones, H. Ghazalibiglar, T.R. Glare, and R.E. Falloon. 2016a. Culturing conditions affect biological control activity of *Trichoderma atroviride* against

Rhizoctonia solani in ryegrass. Journal of Applied Microbiology. 121:461-472. doi:10.1111/jam.13163

Daryaei, A., E.E. Jones, H. Ghazalibiglar, T.R. Glare, and R.E. Falloon. 2016b. Effects of temperature, light and incubation period on production, germination and bioactivity of *Trichoderma atroviride*. Journal of Applied Microbiology. 120:999-1009.

Dell, E.A., D. Bowman, T. Rufty, and W. Shi. 2008. Intensive management affects composition of betaproteobacterial ammonia oxidizers in turfgrass systems. Microb. Ecol. 56:178–190.

Doran, J.W., and M.R. Zeiss. 2000. Soil health and sustainability: Managing the biotic component of soil quality. Appl. Soil Ecol. 15:3–11.

Elliott, M.L., E.A. Guertal, and H.D. Skipper. 2004. Rhizosphere bacterial population flux in golf course putting greens in the southeastern United States. HortScience 39:1754–1758.

Fierer, N., J.A. Jackson, R. Vilgalys, and R.B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71:4117-4120.

Fierer, N., M.A. Bradford, and R.B. Jackson. 2007. Toward an ecological classification of soil bacteria. Ecology 88:1354–1364.

Francis, C.A., K.J. Roberts, J.M. Beman, A.E. Santoro, and B.B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102:14683-14688. doi:10.1073/pnas.0506625102

Gaussoin, R.E., W.L. Berndt, C.A. Dockrell, and R.A. Drijber. 2013. Characterization, development, and management of organic matter in turfgrass systems. In: J.C. Stier, B.P.

Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 425–456.

Habteselassie, M.Y., L. Xu, and J.M. Norton. 2013. Ammonia-oxidizer communities in an agricultural soil treated with contrasting nitrogen sources. Front. Microbiol. 4(326):1-10. doi:10.3389/fmicb.2013.00326

Harman, G.E. 2006. Overview mechanisms and uses of *Trichoderma* spp. Phytopathology. 96:190-194. doi:10.1094/PHYTO-96-0190

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet, and M. Lorito. 2004. *Trichoderma* species—Opportunistic, avirulant plant symbionts. Nat. Rev. 2:43–56. doi:10.1038/nrmicro797

Hatzenpichler, R. 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. Applied and Environ. Microbiol. 78:7501-7510. doi:10.1128/AEM.01960-12

Johnson, P.G., F.S. Rossi, and B.P. Horgan. 2013. Sustainable turfgrass management in an increasingly urbanized world. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 1007–1028.

Kamiya, N., M. Inoue, M. Goto, N. Nakamura, Y. Naruta. 2000. Catalytic and structural properties of surfactant-horseradish peroxidase complex in organic media. Biotechnol. Prog. 16:52-58.

Kandeler, E. 2007. Physiological and biochemical methods for studying soil biota and their function. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 53-83.

Kaye, J.P., R.L. McCulley, and I.C. Burke. 2005. Carbon fluxes, nitrogen cycling, and soil microbial communities in adjacent urban, native and agricultural ecosystems. Glob. Change Biol. 11:575–587. doi:10.1111/j.1365-2486.2005.00921.x

Killham, K., and J.I. Prosser. The prokaryotes. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 119-162.

Kostka, S.J. 2000. Amelioration of water repellency in highly managed soils and the enhancement of turfgrass performance through the systematic application of surfactants. J. Hydrol. 231–232:359–368.

Kuramae, E.E., E. Yergeau, L.C. Wong, A.S. Pijl, J.A. van Veen, and G.A. Kowalchuk. 2010. Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS Microbiol. Ecol. 79:12–24. doi: 10.1111/j.1574-6941.2011.01192.x

Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biol. Biochem. 32:1485–1498.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G.W. Nicol, J.I. Prosser, S.C. Schuster, and C. Schleper. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806-809. doi:10.1038/nature04983

Lickfeldt, D.W., D.S. Gardner, B.E. Branham, and T.B. Voight. 2001. Implications of repeated trinexapac-ethyl applications on Kentucky bluegrass. Agron. J. 93:1164–1168.

Martinez, A., M. Pearce, and L. Burpee. 2017. Turfgrass diseases in Georgia: Identification and control. Univ. Ga. Ext. https://secure.caes.uga.edu/extension/publications/files/pdf/B%201233_7.PDF (accessed 14 May 2018).

Milesi, C., S.W. Running, C.D. Elvidge, J.B. Dietz, B.T. Tuttle, and R.R. Nemani. 2005. Mapping and modeling the biogeochemical cycling of turf grasses in the United States. Environ. Manage. 36:426–438.

Mobley, H.L., and R.P. Hausinger. 1989. Microbial ureases: Significance, regulation, and molecular characterization. Microbiol. Rev. 53:85-108.

Morgan, J.A.W., G.D. Bending, and P.J. White. 2005. Biological costs and benefits to plant-microbe interactions in the rhizosphere. J. Exp. Bot. 56:1729–1739.

Mueller, S.R., and W.R. Kussow. 2005. Biostimulant influences on turfgrass microbial communities and creeping bentgrass putting green quality. HortScience. 40:1904-1910.

Nannipieri, P., E. Kandeler, and P. Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. In: R.G. Burns and R.P. Dick, editors, Enzymes in the environment: Activity, ecology, and applications. Marcel Dekker, New York, NY. p. 1-33.

Nelson, E.B., and C.M. Craft. 2000. Microbial strategies for the biological control of turfgrass diseases. In: J.M. Clark and M.P. Kenna, editors, Fate and management of turfgrass chemicals. ACS Symp. Ser. American Chemical Society, Washington, D.C. p. 342–352.

Norton, J.M. 2011. Diversity and environmental, distribution of ammonia-oxidizing bacteria. In: E.B. Ward, D.J. Arp, and M.G. Klotz, editors. Nitrification. ASM Press, Washington, DC. p. 39-55. doi:10.1128/9781555817145.ch3

Norton, J.M., and J.M. Stark. 2011. Regulation and measurement of nitrification in terrestrial systems. In: M.G. Klotz, editor. Methods in enzymology. Academic Press, Burlington, VA. p. 343-368.

Ouyang, Y., J.M. Norton, J.M. Stark, J.R. Reeve, and M.Y. Habteselassie. 2016. Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. Soil Biol. Biochem. 96:4-15. doi: 10.1016/j.soilbio.2016.01.012

Plante, A.F. 2007. Nitrogen transformations. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 389-432.

Powell, J., and J. Klironomos. 2008. The ecology of plant-microbial mutualisms. In: E.A. Paul, editor. 2008. Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 257–281.

Qian, Y.L., and M.C. Engelke. 1999. Influence of trinexapac-ethyl on diamond zoyiagrass in a shade environment. Crop Sci. 39:202–208.

Reicher, Z.J., P.H. Dernoeden, and D.S. Richmond. 2013. Insecticides, fungicides, herbicides, and growth regulators used in turfgrass systems. In: J.C. Stier, B.P. Horgan, and S.A. Bonos, editors, Turfgrass: Biology, use, and management. Agron. Monogr. 56. ASA, SSSA, and CSSA, Madison, WI. p. 891–932.

Rotthauwe, J., K. Witzel, and W. Leisack. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63:4704-4712.

Savazzini, F., C.M.O. Longa, and I. Pertot. 2009. Impact of biocontrol agent *Trichoderma atroviride* SC1 on soil microbial communities of a vineyard in northern Italy. Soil Biology and Biochemistry. 41:1457-1465. doi:10.1016/j.soilbio.2009.03.027

Schlesinger, W.H., and J.A. Andrews. 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48:7-20.

Schmidt, M.W.I., Torn, M.S., S. Abiven, T. Dittmar, G. Guggenberger, I.A. Janssens, M. Kleber, I. Kögel-Knabner, J. Lehmann, D.A.C. Manning, P. Nannipieri, D.P. Rasse, S. Weiner, and S.E. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature. 478:49–56. doi:10.1038/nature10386

Shi, W., E. Dell, D. Bowman, and K. Iyyemperumal. 2006. Soil enzyme activities and organic matter composition in a turfgrass chronosequence. Plant Soil 288:285–296. doi:10.1007/s11104-006-9116-1

Smucker, A.J.M. and Saettler, A.W. 1977. Influence of soil nitrogen, soil oxygen, and soil water stresses upon ozone damage to *Phaseolus vulgaris* [abstract only]. Proc. Amer. Phytopathol. Soc. 4.

Stier, J.C., J.N. Rogers, III, J.R. Crum, and P.E. Rieke. 1999. Flurprimidol effects on Kentucky bluegrass under reduced irradiance. Crop Sci. 39:1423–1430.

Strandberg, M., K. Blombäck, A.M. Dahl Jensen, and J.W. Knox. 2012. Priorities for sustainable turfgrass management: A research and industry prospective. Acta Agric. Scand., Sect. B. 62:3–9.

Tabatabai, M.T. 1994. Soil enzymes. In: R.W. Weaver, J.S. Angel, P.S. Bottomley, editors, Methods of Soil Analysis—Part 2: Microbiological and Biochemical Properties. SSSA Book Ser. No. 5, SSSA, Madison, WI. p. 775-833.

Treusch, A.H., S. Leininger, A. Kletzin, S.C. Schuster, H. Klenk, and C. Schleper. 2005. Novel genes for nitrite reductase and *Amo*-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environ. Microbiol.7:1985-1995. doi:10.1111/j.1462-2920.2005.00906.x

van Bruggen, A.H.C., and A.M. Semenov. 2000. In search of biological indicators for soil health and disease suppression. Appl. Soil Ecol. 15:13–24.

van der Heijden, M.G.A., R.D. Bardgett, and N.M. van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296–310. doi:10.1111/j.1461-0248.2007.01139.x

Verma, M., S.K. Brar, R.D. Tyagi, R.Y. Surampalli, and J.R. Valéro. 2007. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochemical Engineering Journal. 37:1-20. doi:10.1016/j.bej.2007.05.012

Voroney, R.P. 2007. The soil habitat. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 25-49.

Wong, P.T.W., and J.H. McBeath. 1999. Plant protection by cold-adapted fungi. In: R. Margesin, and F. Schinner, editors, Biotechnological applications of cold-adapted organisms. Springer, Berlin, Heidelberg. p.177-190.

Wyngaard, N., D.H. Franklin, M.Y. Habteselassie, A. Mundepi, and M.L. Cabrera. 2016. Legacy effect of fertilization and tillage systems on nitrogen mineralization and microbial communities. 80:1262-1271. doi: doi:10.2136/sssaj2016.03.0070

Yao, H., Z. He, M.J. Wilson, and C.D. Campbell. 2000. Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microb. Ecol. 40:223–237. doi:10.1007/s002480000053

Zhang, X.Z., E.H. Ervin, and R.E. Schmidt. 2003. Plant growth regulators can enhance the recovery of Kentucky bluegrass sod from heat injury. Crop Sci. 43:952–956.

Table 3.1. Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and control (NTC) treatments applied to experimental plots in the putting green trial.

Treatment	Product type	Trade name	Manufacturer	Active ingredient (a.i.)	Rate of a.i. application
					—L a.i. ha^{-1}—
Rev	Wetting agent	Revolution	Aquatrols	Modified alkylated polyol	9.6
C+D	Wetting agent	Cascade Plus	Precision Laboratories	Alcohol ethoxylates	1.3
				Polyethylene polypropylene glycol block copolymer	11
	Wetting agent	Duplex	Precision Laboratories	Alcohol ethoxylates	0.11
				Alkyl aryl sulfonate	0.02
				Ethylenediaminetetraacetic acid	0.05
					—g a.i. ha^{-1}—
PH	Microbial inoculant	Plant Helper	AmPac, Inc.	*Trichoderma atroviride*	56
NTC	-	-	-	-	-

Table 3.2. PrimoMaxx (PM), Cutless MEC (CL) and control (NTC) treatments applied to experimental plots in the fairway trial.

Treatment	Product type	Trade name	Manufacturer	Active ingredient (a.i.)	Rate of a.i. application
					—L a.i. ha^{-1}—
PM	PGR	PrimoMaxx	Syngenta	Trinexapac-ethyl	0.13
CL	PGR	Cutless MEC	SePRO	Flurprimidol	0.29*
NTC	-	-	-	-	-

*Rate of a.i. application in CL plots 0 DAT was 0.21 L a.i. ha^{-1}.

Table 3.3. Target genes, amplicon lengths, primer sequences, and thermal cycling conditions used in qPCR analyses in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), PrimoMaxx (PM), Cutless MEC (CL), and non-treated control (NTC) soil samples collected 0 to 5 cm from the soil surface.

Target group	Gene	Amplicon length (bp)	Primers		Thermocycling conditions	References
			Name	Sequence		
Total bacteria	16S rDNA	200	Eub338	5'-ACTCCTACGGGAGGCAGCAG-3'	95ºC for 15 min; 40 cycles of 95ºC for 1 min, 53ºC for 1 min, and 72ºC for 1 min	(Fierer et al., 2005)
			Eub518	5'-ATTACCGCGGCTGCTGG-3'		
Total fungi	18s rDNA	422	nu-SSU-0817	5'-TTAGCATGGAATAATRRAATAGGA-3'	94ºC for 10 min; 40 cycles of 94ºC for 1 min, 56ºC for 1 min, and 72ºC for 2 min	(Borneman and Hartin, 2000)
			nu-SSU-1196	5'-TCTGGACCTGGTGAGTTTCC-3'		
AOA	Archaeal amo*A*	635	Arch*amoA*F	5'-TTATGGTCTGGCTTAGACG-3'	95ºC for 10 min; 40 cycles of 95ºC for 1 min, 56ºC for 1 min, and 72ºC for 3 min	(Francis et al., 2005; Wynngaard et al., 2016)
			Arch*amoA*R	5'-GCGGCCATCCATCTGTATGT-3'		
AOB	Bacterial amo*A*	491	amoA-1F	5'-GGGGTTTCTACTGGTGGT-3'	95ºC for 10 min; 40 cycles of 95ºC for 1 min, 57ºC for 1 min, and ºC for 3 min	(Rotthauwe et al., 1997; Wynngaard et al., 2016)
			amoA-2R	5'-CCCCTCGGGAAAGCCTTCTTC -3'		

Table 3.4. Summary statistics of microbial abundance, enzyme activities, and soil respiration responses in the putting green soil treated with Cascade and Duplex (C+D), Revolution (Rev), and PlantHelper (PH), and fairway soil treated with PrimoMaxx (PM), Cutless MEC (CL).

	Putting green					Fairway				
Response	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
Total bacteria* (copy g^{-1})	5.1×10^{7}	3.8×10^{7}	4.3×10^{6}	3.7×10^{5}	1.9×10^{7}	3.2×10^{7}	1.2×10^{7}	3.0×10^{7}	1.0×10^{7}	5.7×10^{7}
Total fungi* (copy g^{-1})	1.3×10^{5}	1.2×10^{4}	1.1×10^{4}	4.6×10^{2}	6.0×10^{5}	4.4×10^{4}	2.6×10^{4}	3.9×10^{4}	1.2×10^{4}	1.4×10^{5}
AOA* (copy g^{-1})	2.2×10^{3}	1.5×10^{3}	2.1×10^{3}	1.3×10^{2}	6.8×10^{3}	7.0×10^{3}	4.6×10^{3}	5.5×10^{3}	7.9×10^{2}	2.2×10^{4}
AOB* (copy g^{-1})	6.0×10^{4}	5.0×10^{4}	4.7×10^{4}	2.7×10^{3}	2.0×10^{5}	5.2×10^{5}	4.1×10^{5}	4.0×10^{5}	1.5×10^{4}	2.0×10^{6}
Phosphatase activity** (µmol P*i*—g^{-1},h^{-1})	52	43	36	0.11	198	366	225	320	35	1,059
Urease activity** (µmol NH_3—g^{-1},h^{-1})	1.5	1.5	1.5	0	6.2	34	18	31	8.4	130
Soil respiration** (mg CO_2 g^{-1})	0.13	0.05	0.12	0.06	0.35	0.43	0.45	0.31	0	1.8

* = samples from top 5 cm of soil profile; ** = samples from top 10 cm of soil profile

Table 3.5. Mixed model analysis of microbial abundance in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and non-treated control (NTC) soil samples collected from the top 5 cm in the putting green.

		Main effect *p*-value (α=0.05)			
Response	R^2*	Treatment	Sampling date	Treatment×sampling date	Experimental plot
Total bacteria (copy g^{-1})	0.340	0.0043	0.5294	0.3215	0.9039
Total fungi (copy g^{-1})	0.297	0.0764	0.0035	0.3927	0.9855
AOA (copy g^{-1})	0.440	0.7341	0.0020	0.2379	0.2751
AOB (copy g^{-1})	0.516	0.0021	0.0144	0.1843	0.5397

* = R^2 values indicate the fit of overall model describing response data.

Table 3.6. Microbial abundance in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and non-treated control (NTC) soil samples collected from the top 5 cm in the putting green 0 to 84 days after treatment.

	Mean microbial population (copy g^{-1})			
Treatment	Total bacteria	Total fungi	AOA	AOB
C+D	4.3×10^{6}b	1.1×10^{4}a	2.1×10^{3}a	5.3×10^{4}b
Rev	4.6×10^{6}b	9.1×10^{3}a	2.1×10^{3}a	3.1×10^{4}b
PH	3.1×10^{6}b	1.3×10^{4}a	2.0×10^{3}a	4.9×10^{4}b
NTC	8.2×10^{6}a	1.9×10^{4}a	2.6×10^{3}a	1.1×10^{5}a

Tukey's HSD among treatments separated by microbial population are indicated by different letters (α=0.05)

Table 3.7. One-way ANOVA comparing microbial abundance in PrimoMaxx (PM), Cutless MEC (CL), and non-treated control (NTC) soil samples collected from the top 5 cm in the fairway.

	Response			
	Total bacteria (copy g^{-1})		AOA (copy g^{-1})	
DAT	R^2*	Treatment effect *p*-value (α=0.05)	R^2	Treatment effect *p*-value (α=0.05)
0	0.124	0.4522	0.203	0.2558
15	0.179	0.3058	0.140	0.4027
42	0.628	0.0027	0.034	0.8106
63	0.037	0.7977	0.236	0.1978
84	0.220	0.2251	0.059	0.6960

* = R^2 values indicate the fit of overall model describing response data.

Table 3.8. Mixed model analysis of microbial abundance in PrimoMaxx (PM), Cutless MEC (CL), and non-treated control (NTC) soil samples collected from the top 5 cm in the fairway.

		Main effect *p*-value (α=0.05)			
Response	R^2*	Treatment	Sampling date	Treatment×sampling date	Experimental plot
Total fungi (copy g^{-1})	0.074	0.3415	0.1217	0.1689	0.8030
AOB (copy g^{-1})	0.254	0.9249	<0.0001	0.1644	0.9530

* = R^2 values indicate the fit of overall model describing response data.

Table 3.9. Total bacteria abundance in PrimoMaxx (PM), Cutless MEC (CL), and non-treated control (NTC) soil samples collected from the top 5 cm in the fairway 0 to 98 days after treatment (DAT).

	Mean total bacteria (copy g^{-1})				
Treatment	0 DAT	15 DAT	42 DAT	63 DAT	98 DAT
PM	4.1×10^{7}a	2.8×10^{7}a	4.1×10^{7}a	2.9×10^{7}a	3.1×10^{7}a
CL	3.7×10^{7}a	3.3×10^{7}a	2.1×10^{7}ab	3.5×10^{7}a	4.1×10^{7}a
NTC	3.8×10^{7}a	2.3×10^{7}a	3.0×10^{7}b	3.0×10^{7}a	4.1×10^{7}a

Tukey's HSD among treatments separated by DAT are indicated by different letters (α=0.05).

Table 3.10. Mixed model analysis of phosphatase and urease activities in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and non-treated control (NTC) soil samples collected from the top 5 cm in the putting green 0 to 84 days after treatment (DAT) and PrimoMaxx (PM), Cutless MEC (CL), and NTC soil samples collected from the top 5 cm of the fairway 0 to 98 DAT.

	Main effect *p*-value (α=0.05)			
	Putting green		Fairway	
Parameter	Rate of phosphatase activity	Rate of urease activity	Rate of phosphatase activity	Rate of urease activity
R^2*	0.662	0.511	0.417	0.420
Treatment	0.7443	0.0005	0.2386	0.0426
Soil depth	<0.0001	<0.0001	<0.0001	<0.0001
Sampling date	<0.0001	0.0670	<0.0001	0.0005
Treatment × soil depth	0.3462	0.7977	0.1467	0.0032
Treatment × sampling date	0.1891	0.0006	0.2038	0.1160
Soil depth × sampling date	<0.0001	0.2837	0.0085	0.5692
Treatment × soil depth × sampling date	0.9129	0.0313	0.1488	0.9944
Experimental plot	0.5791	0.0157	0.6622	0.0677

* = R^2 values indicate the fit of the overall model describing response data.

Table 3.11. Urease activity (µmol NH_3 evolved—$g^{-1} \cdot h^{-1}$) in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and non-treated control (NTC) soil samples collected 0 to 10 cm from the putting green after 24-h incubation at 22ºC.

	Mean urease activity (µmol NH_3 evolved—$g^{-1} \cdot h^{-1}$)					
	0 DAT		14 DAT		84 DAT	
Treatment	0 to 5 cm	5 to 10 cm	0 to 5 cm	5 to 10 cm	0 to 5 cm	5 to 10 cm
C+D	3.5^{a}	0.0^{b}	1.7^{a}	0.6^{b}	4.4^{a}	3.1^{ab}
Rev	2.8^{a}	0.2^{b}	1.5^{a}	0.4^{b}	1.8^{abc}	0.9^{bcd}
PH	2.7^{a}	0.0^{b}	1.1^{a}	1.0^{b}	2.3^{ab}	0.4^{cd}
NTC	2.8^{a}	0.6^{b}	1.4^{a}	0.6^{b}	2.8^{ab}	0.0^{d}

Tukey's HSD among treatments separated by DAT are indicated by different letters (α=0.05).

Table 3.12. Urease activity (µmol NH_3 evolved—$g^{-1}\cdot h^{-1}$) in PrimoMaxx (PM), Cutless MEC (CL), and non-treated control (NTC) soil samples collected 0 to 10 cm from the fairway after 24-h incubation at 22ºC.

	Mean urease activity (µmol NH_3 evolved—$g^{-1}\cdot h^{-1}$)	
	Soil depth	
Treatment	0 to 5 cm	5 to 10 cm
PM	43ab	23a
CL	35b	26a
NTC	51a	23a

Tukey's HSD among treatments separated by soil depth are indicated by different letters (α=0.05).

Table 3.13. Mean soil respiration (mg CO_2 g^{-1}) in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and non-treated control (NTC) soil samples collected 0 to 10 cm from the putting green after 24-h incubation at 22ºC.

	Mean soil respiration (mg CO_2 g^{-1})		
Treatment	0 DAT*	14 DAT*	84 DAT*
C+D	0.12b	0.11a	0.18a
Rev	0.17a	0.09a	0.09a
PH	0.17a	0.09a	0.13a
NTC	0.13b	0.14a	0.14a
R^2*	0.657	0.321	0.191
p-value*	0.0384	0.3515	0.6161

LSD among treatments separated by DAT are indicated by different letters (α=0.05).
*R^2 and *p*-values represent the fit of one-way ANOVA models and the significance of treatment effect on soil respiration response.

Table 3.14. Mean soil respiration (mg CO_2 g^{-1}) in PrimoMaxx (PM), Cutless MEC (CL), and non-treated control (NTC) soil samples collected 0 to 10 cm from the fairway soil after 24-h incubation at 22ºC.

	Mean soil respiration (mg CO_2 g^{-1})				
Treatment	0 DAT	15 DAT	42 DAT	63 DAT	98 DAT
PM	0.02b	0.72ab	0.27a	0.41a	0.36a
CL	0.32a	0.17b	0.24a	0.76a	0.31a
NTC	0.39a	1.65a	0.17b	0.21a	0.39a
R^2*	0.911	0.716	0.774	0.380	0.055
p-value*	0.0023	0.0229	0.0116	0.2385	0.8431

LSD among treatments separated by DAT are indicated by different letters (α=0.05).
*R^2 and *p*-values represent the fit of one-way ANOVA models and the significance of treatment effect on soil respiration response.

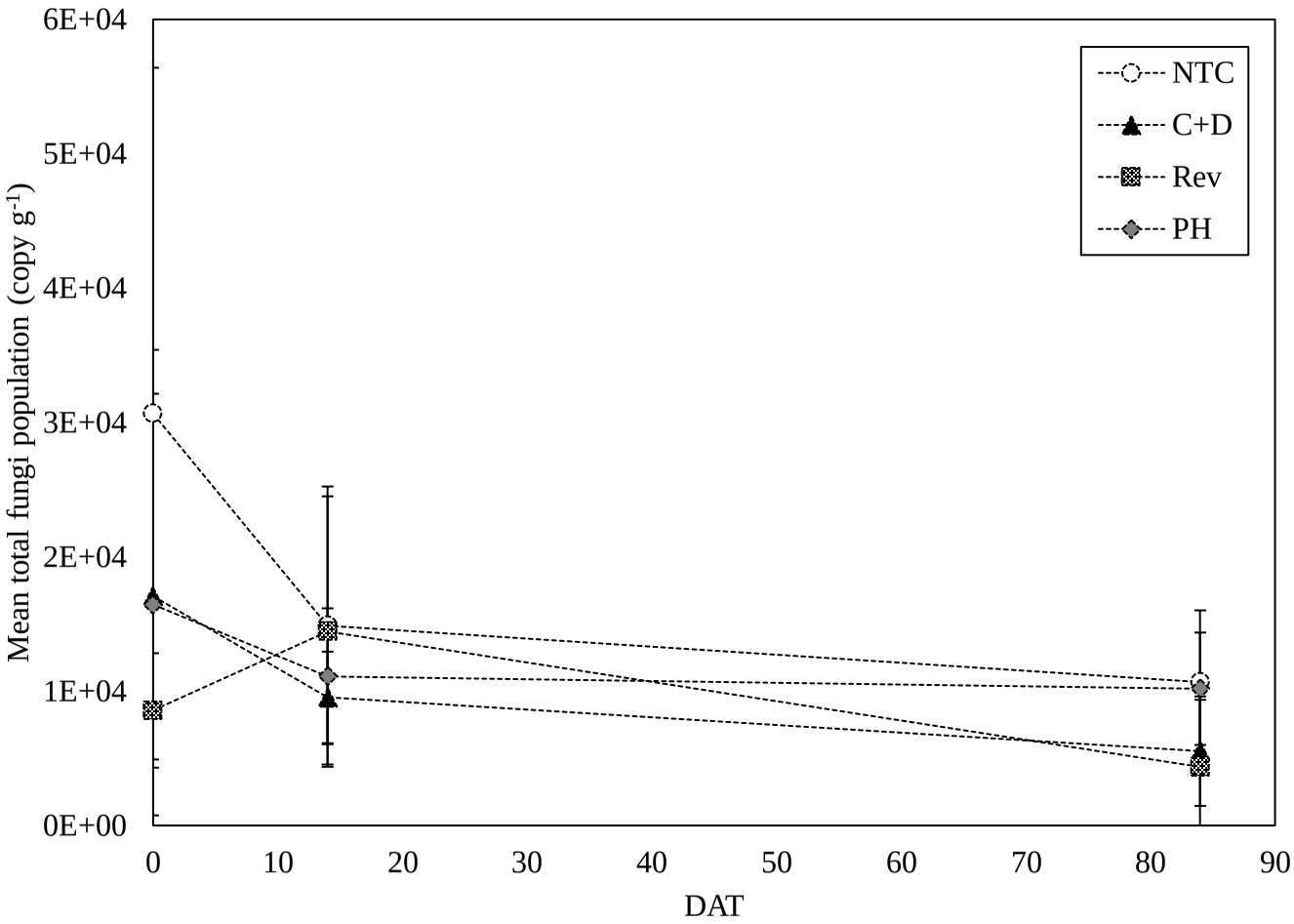

Figure 3.1. Mean ± SD total fungi abundance (copy g^{-1}) in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and non-treated control (NTC) samples collected 0 to 5 cm from the putting green 0 to 84 days after treatment (DAT).

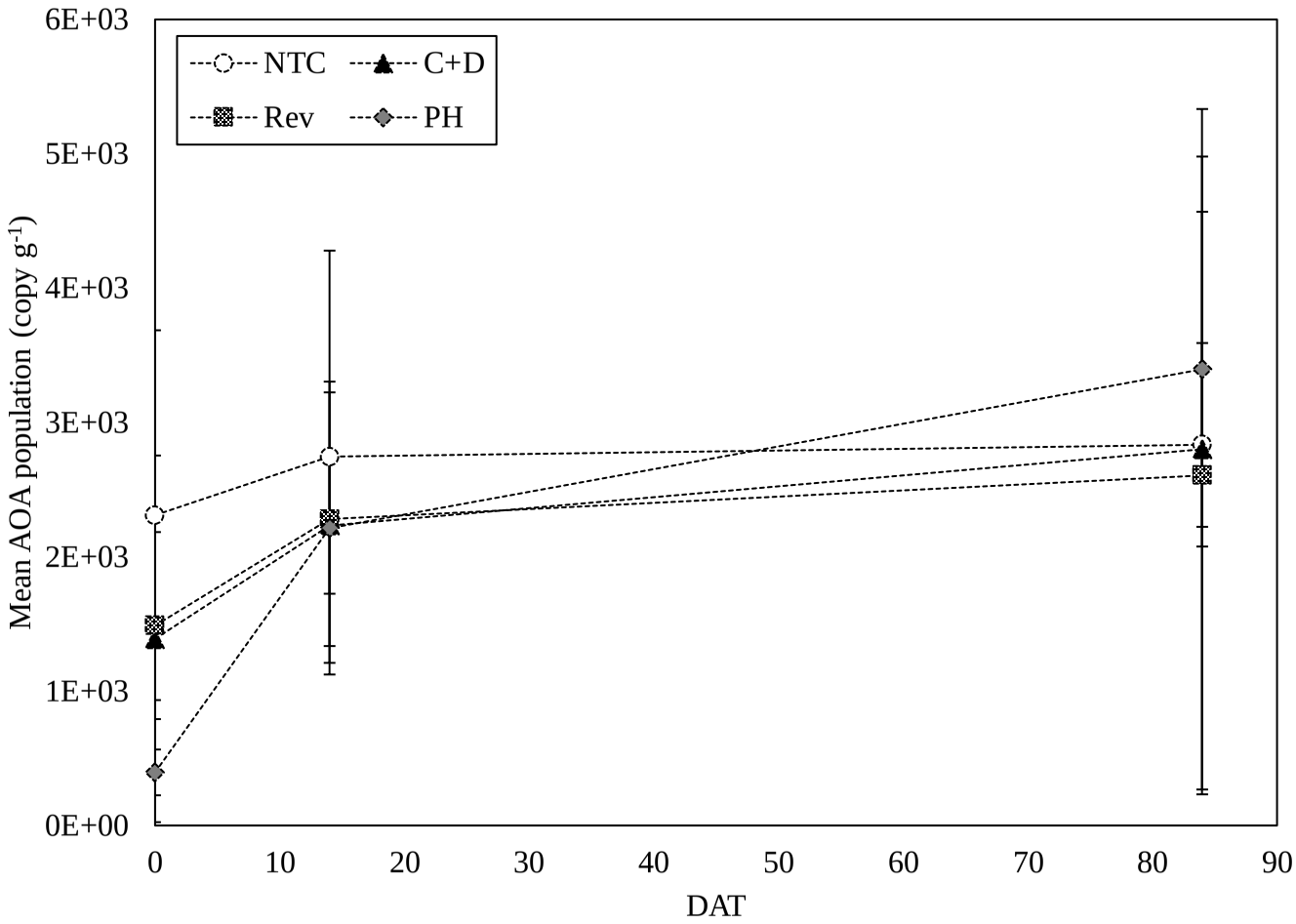

Figure 3.2. Mean ± SD AOA abundance (copy g^{-1}) in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and non-treated control (NTC) samples collected 0 to 5 cm from the putting green 0 to 84 days after treatment (DAT).

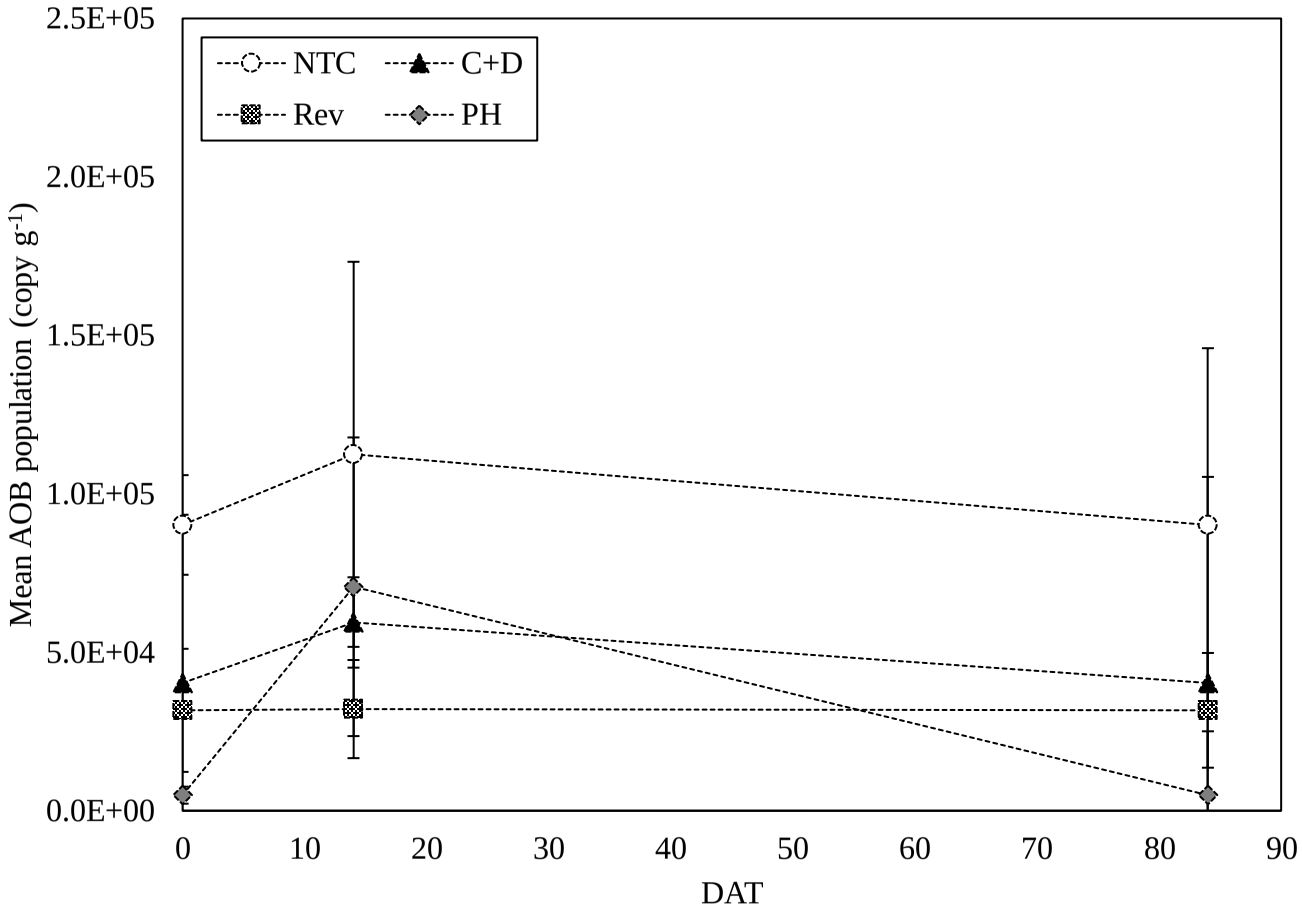

Figure 3.3. Mean ± SD AOB abundance (copy g^{-1}) in Cascade and Duplex (C+D), Revolution (Rev), PlantHelper (PH), and non-treated control (NTC) samples collected 0 to 5 cm from the putting green 0 to 84 days after treatment (DAT).

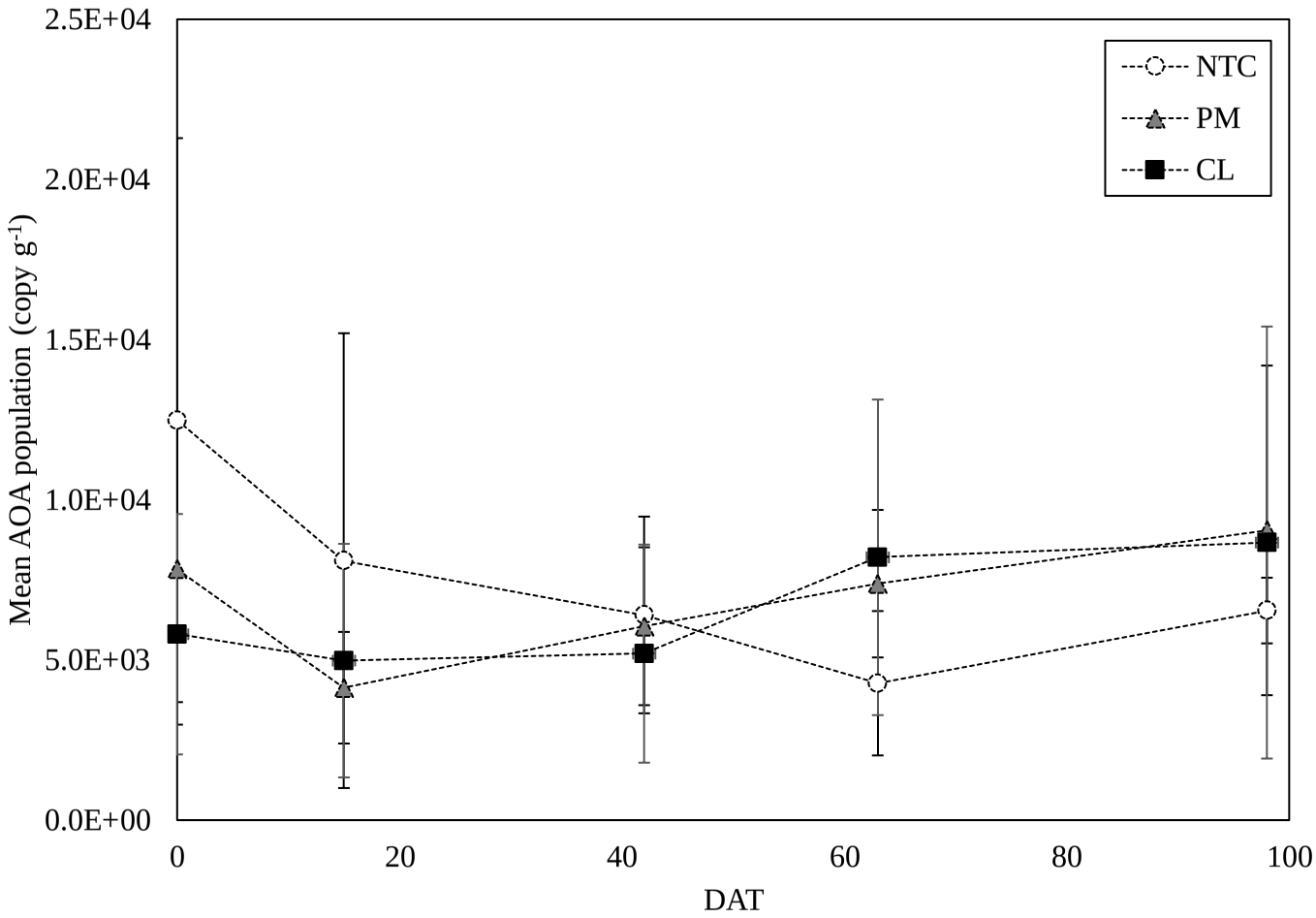

Figure 3.4. Mean ± SD AOA abundance (copy g^{-1}) in PrimoMaxx (PM), Cutless MEC (CL), and non-treated control (NTC) samples collected 0 to 5 cm from the fairway 0 to 98 days after treatment (DAT).

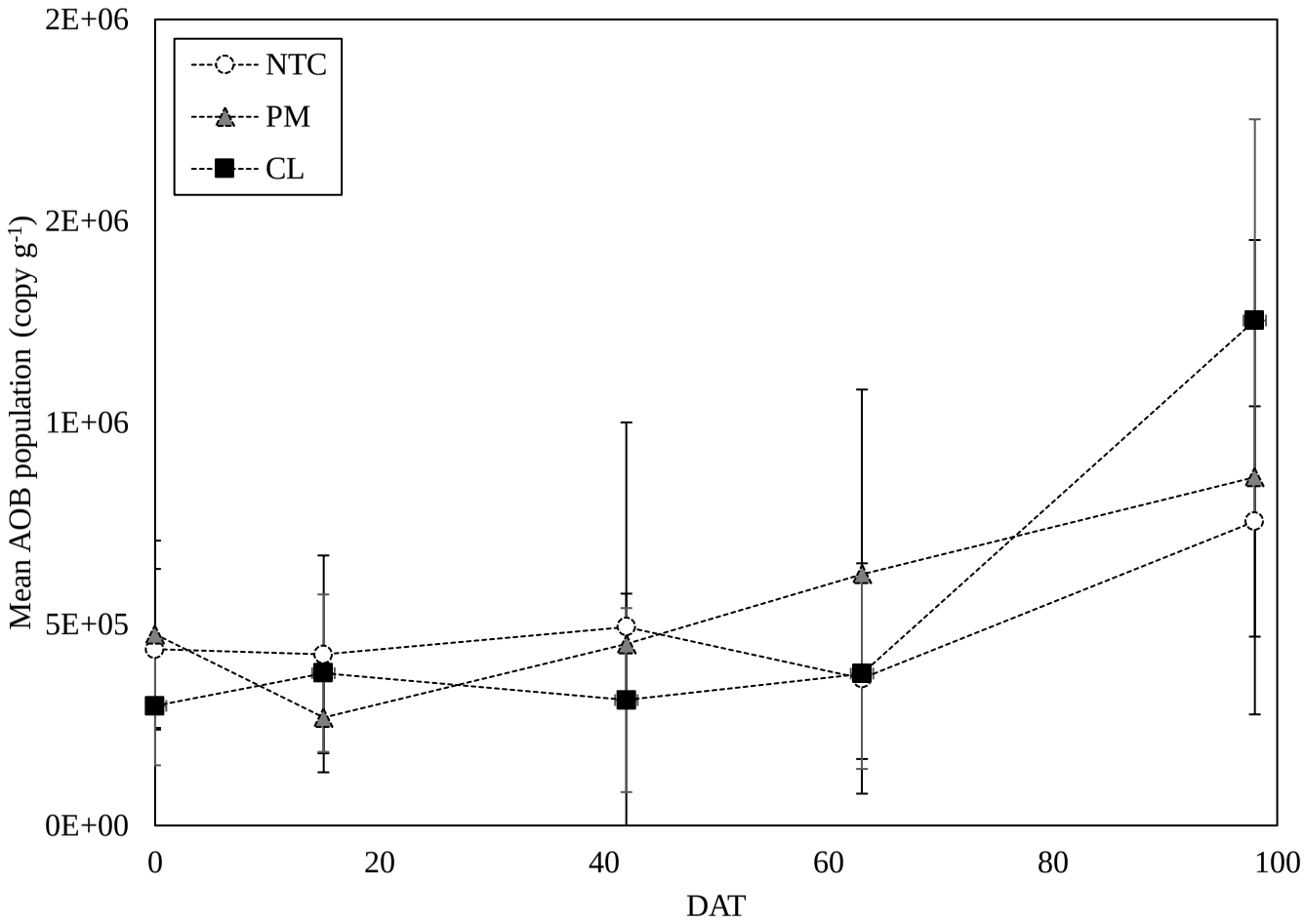

Figure 3.5. Mean ± SD total AOB abundance (copy g^{-1}) in PrimoMaxx (PM), Cutless MEC (CL), and non-treated control (NTC) samples collected 0 to 5 cm from the fairway 0 to 98 days after treatment (DAT).

CHAPTER 4

SOIL TEMPERATURE AND MOISTURE EFFECTS ON *TRICHODERMA ATROVIRIDE* IN GEORGIA SOIL[2]

[2] Diera, A. A., Habteselassie, M.Y., Cabrera, M.L., and Raymer P.L. To be submitted to *Applied Soil Ecology*

ABSTRACT

Fungi from the *Trichoderma* spp. genera are becoming of increasing interest in turfgrass management for their parasitization of phytopathogens and enhancement of turf resiliency. The fungal strain *Trichoderma atroviride* Karsten CHS 861 originated from subarctic soils with a temperature range of 4ºC to 33ºC and is the active ingredient in the microbial inoculant Plant Helper. Because the viability of *T. atroviride* CHS 861 has not been evaluated in Georgia soil conditions, a lab study was designed to determine soil temperature and moisture effects for *T. atroviride* by simulating a range of soil conditions across a temperature gradient (11ºC, 24ºC, and 32ºC) and two levels of soil moisture (23%±3.7%, 51%±1.7%) over a 57-d incubation period. A Georgia sandy loam soil was sterilized and inoculated with *T. atroviride* CHS 861 to observe behavior uninhibited by a native soil microbial community. Half of the remaining non-sterile soil was treated with the *T. atroviride* CHS 861 inoculant to observe performance in a competitive, natural soil environment, while the remaining nonsterile soil served as negative controls. *T. atroviride* abundance was analyzed using ANCOVA, and Kruskal-Wallis rank-sum tests were conducted to identify the effects of soil temperature and moisture on growth rates ($k \cdot d^{-1}$). Results demonstrated that *T. atroviride* CHS 861 was the most competitive at 11ºC at 23% soil moisture. *T. atroviride* CHS 861 growth rates were comparable among temperatures but exhibited significant a negative correlation with soil moisture in sterile, inoculated samples. Abundance data also pointed to the sensitivity of the inoculant to irrigation and rainfall events. *T. atroviride* CHS 861 may be most suited to establish itself in soil when applied in early to late spring in Georgia.

INTRODUCTION

Many turfgrass managers are incorporating biological control agents as an alternative to chemical fungicides to suppress disease and improve turfgrass resiliency (Christians et al., 2016; Nelson 1997; Aamlid et al., 2012; Boulter et al., 2000). *Trichoderma* spp. are saprophytic, fast-growing fungi commonly applied as biological control agents, because they are well-known parasites to phytopathogens and are ubiquitous in diverse environments around the world (Harman et al., 2004; Harman, 2006; Verma et al., 2007; Klein and Eveleigh, 1998). *Trichoderma* spp. are beneficial in soil communities, because they regulate the presence of certain pathogenic fungi through specialized methods including encoding antifungal enzymes into plant root tissue and mycoparasitism (Klein and Eveleigh, 1998; Harman et al., 2006; Schimel, 2007; Benítez et al., 2004). Encoding antifungal enzymes into root tissue involves a direct relationship between host plants and *Trichoderma* spp. that induces plant resistance to disease (Harman et al., 2006; Benítez et al., 2004). *Trichoderma* spp. infect the plant root epidermis and secrete antifungal, cell wall-degrading enzymes into the root tissue such as chitinase, β-1,3 glucanase, and proteases (Harman et al., 2004; Benítez et al., 2004). By using these enzymes, both *Trichoderma* spp. and host plants can suppress pathogens through mycoparasitism. Mycoparasitism involves the use of cell wall-degrading enzymes to penetrate, infect, and destroy host pathogens (Verma et al., 2007; Schimel, 2007). *Trichoderma* spp. also influences disease suppression through routine nutrient and habitat competition (Benítez et al., 2004). The pervasiveness of the genus is a testament to the robust and resourceful characteristics of *Trichoderma* spp.

The survivability of *Trichoderma* spp. varies widely among the genera, thus biological control activity vary among each species and strain due to a variety of conditions. *Trichoderma*

atroviride Karsten is one species of interest in the turf industry for its suppression of common turfgrass diseases like *Sclerotinia homoeocarpa* Benn. (dollar spot), *Rhizoctonia solani* Kühn (brown patch), and *Pythium* spp. Pringsh. (Pythium root rot) among many others (Martinez et al., 2017; Gómez-Rodríguez et al., 2018; Nawrocka et al., 2018). *T. atroviride* has a variety of applications and has recently demonstrated disease control in cucumber production, nematode suppression in pineapple production, and disease control when applied to organic waste material (Nawrocka et al., 2018; Kiriga et al., 2018; Kowalska et al., 2017). Recent research has observed shortened life spans of *T. atroviride* LU132 in culture conditions, a 20-d conidium production cycle, optimal germination and bioactivity in 30ºC, and ideal carbon-to-nitrogen ratio (C:N), water activity level (a_w) and pH as 5:1, 0.961, and 7.5 respectively (Daryaei et al., 2016a; 2016b; 2016c; 2016d).

The strain *T. atroviride* CHS 861 is native to the sub-arctic region of Alaska and is the active ingredient in PlantHelper (AmpPac Biotech, Fresno, CA), a product recommended for biological control of turfgrasses diseases. The psychrophilic fungus grows within a temperature range of 4 to 33ºC which exceeds the temperature range of some of its target pathogens including *R. solani* (1 to 32ºC) and *Pythium* spp. (7 to 30ºC) (Wong and McBeath, 1999). Research on *T. atroviride* CHS 861 is scarce, although one study observed total inhibition of *Phytophthora ramorum* (sudden oak death) on shrub leaves (Elliott et al., 2009).

Studies evaluating the application of *T. atroviride* CHS 861 in Georgia were not found in the literature. Although *T. atroviride* CHS 861 is native to a subarctic environment, the inoculant has exhibited a wide range of survivability temperatures. Thus, this study was designed to examine the behavior of *T. atroviride* CHS 861 in simulated soil conditions under laboratory conditions that reflect temperatures and rainfall events that are characteristic of the growing

season in Georgia. The objective of this study was to determine optimal soil temperature and moisture conditions for the *T. atroviride* CHS 861 from the product PlantHelper using a sandy loam soil from Georgia. *T. atroviride* abundance was expected to be comparable across three temperatures (11ºC, 24ºC, and 32ºC) and greater in lower soil moisture levels of 23% than 51%. *T. atroviride* growth rate was expected to positively correlate with temperature and decline with increasing soil moisture levels.

MATERIALS AND METHODS

Inoculant preparation

The product Plant Helper described in Chapter 3 and labeled to contain 3.0×10^8 CFU g^{-1} was obtained in November 2017. Standard culture methods were used to cultivate a pure strain of the active ingredient *T. atroviride* from the product. Sterile phosphate buffer solution (pH 7.0) and petri dishes with rose bengal glucose media were prepared. One gram of Plant Helper was diluted to 1:10 with 9 mL of phosphate buffer solution in a 16-mL polystyrene vial and shaken on a rotary shaker for 1 h. One hundred microliters of the contents in the vial were transferred to a Rose Bengal-glucose plate and allowed to incubate inverted at 30ºC for 4 d. Uniform colonies characteristic of *T. atroviride* were picked based on their known colony morphology and color (Siddiquee, 2017) and added to separate Erlenmeyer flasks with 100 mL of rose bengal glucose broth. These samples were allowed to incubate at 30ºC for 4 d to create pure culture strains of *T. atroviride*. Pure *T. atroviride* strains were added to 1.05 L of phosphate buffer solution for the inoculant treatment.

Treatment preparation

A sandy loam soil with 5.5 pH (measured in 0.01 M $CaCl_2$) was obtained for this experiment. Six soil treatments were prepared as follows. A sterilized soil treatment served as a

positive control for the presence of pure *T. atroviride*; 1 kg of soil was autoclaved and divided evenly into two Ziploc bags. The appropriate volumes of *T. atroviride* inoculant suspensions were added to each bag to achieve an average of 23% and 51% water content treatments. Two non-sterile soil treatments were similarly prepared and divided evenly among four Ziploc bags. The same volumes of liquid corresponding to 23% and 51% soil moisture were added to the bags, but two received the inoculant solution, and sterile water was added to the other two bags to serve as negative controls. All bags were mixed thoroughly to ensure homogenous distribution. Six 40-g soil samples from each Ziploc bag were measured in plastic cups. Four replicates of each combination of sterility, inoculant presence, and soil moisture underwent a 5-d pre-incubation period in separate incubators at 11ºC, 24ºC, and 32ºC before sampling was started. Soil moisture was monitored regularly and adjusted gravimetrically as needed. The mean soil moisture levels across the scope of the trial ranged from 23%±3.7% and 51%±1.7%.

T. atroviride enumeration

Standard culture methods were used to quantify fungal CFUs 5, 12, 19, 26, and 57 DAI. One gram of soil was diluted in 9 mL of sterile phosphate buffer solution (pH 7.0) in 16-mL polystyrene vials and shaken on a rotary shaker for 1 h. Contents of each vial were diluted further in sterile 96-multiwell plates using phosphate buffer solution. One hundred microliters were transferred to rose bengal glucose medium, and final dilutions from the vial contents varied from 10^{-5} to 10^{-2} depending on previously collected data. Petri dishes were inverted and allowed to incubate in the dark at 30ºC for 3 to 4 d. Colony-forming units (CFUs) of all fungi and *T. atroviride* were counted, and equation (7) was used to estimate both populations (CFU g^{-1}) in each petri dish.

$$\text{CFU } g^{-1} = \frac{\text{CFU} \times \text{dilution factor}}{\text{dry soil weight (g)}} \quad (7)$$

The absence of *T. atroviride* in all nonsterile soil samples without the inoculant confirmed *T. atroviride* from the product was not present in the positive control or inoculated nonsterile soil. The ratio between *T. atroviride* abundance to unidentified fungi in the nonsterile soil samples was calculated for each sample per sampling day using equation (8).

$$\%\ T.\ atroviride \text{ to unidentified fungi} = \left[\frac{T.\ atroviride}{(\text{Unidentified fungi- } T.\ atroviride)}\right] \times 100 \quad (8)$$

The derived ratios were averaged for each temperature and soil moisture condition.

T. atroviride population estimates (y) were fitted against time (x) to determine the daily growth rate in each soil sample. The exponential model was used to determine the fungal growth rate (k d^{-1}) in sterile and nonsterile samples using equation (9) where y, t, and a represented abundance, DAI, and starting inoculant abundance respectively.

$$k\ \text{d}^{-1} = -\left[\frac{ln\left(\frac{y}{a}\right)}{t}\right] \quad (9)$$

Statistical analysis

To address the pseudoreplication of initial soil treatments, sterile and non-sterile soil data were separated as analyzed in JMP Pro 13 as follows. Mean abundance, % *T. atroviride* to unidentified fungi, and k d^{-1} values calculated for each cluster of data separated by temperature, soil moisture, and DAI. Mean abundance in sterile and non-sterile soils and mean % *T. atroviride* to unidentified fungi were fitted to ANCOVA models using temperature, soil moisture, and the interaction between temperature and soil moisture as categorical variables and DAI as a covariate. The ANCOVA model for sterile abundance violated the equal variance assumption, and the remaining models violated the normality assumption. All response data were transformed by adding 1 to each mean value and conducting a log transformation. Tukey's HSD was used to conduct post-hoc analyses. The k d^{-1} data in both sterile and nonsterile soils

could not be transformed to satisfy ANCOVA assumptions, and Kruskal-Wallis rank-sum tests were conducted to analyze temperature and soil moisture effects separately.

RESULTS AND DISCUSSION

Trichoderma atroviride abundance in sterile soil

T. atroviride abundance varied among the three temperature conditions in both sterile and nonsterile soils contrary to the initial hypothesis stating abundance would be comparable among them (Table 4.1). However, results in both soils supported the second prediction anticipating higher *T. atroviride* abundance under 53% soil moisture compared to 23%.

In the sterile soil, the highest mean abundance of *T. atroviride* over the duration of the study was observed in the samples incubating at 24ºC with a soil moisture range of 23%. The lowest mean abundance of *T. atroviride* occurred in the samples incubating at 11ºC with a soil moisture range of 52%. The ANCOVA model identified significant differences in mean abundance among temperature levels, between soil moisture conditions, and within the interaction of the two predictors (Table 4.2). Uninhibited by a soil microbial community, *T. atroviride* populations grew the most under the moderately warm temperature of 24ºC and lower soil moisture conditions (23%) from 1.6×10^5 CFU g^{-1} to 1.4×10^6 CFU g^{-1} over 57 d (Figure 4.1). However, Tukey's HSD detected that abundance among almost all soil conditions were comparable, indicating *T. atroviride* CHS 861 exhibits potential to grow to comparable population levels across the temperature gradient and two soil moisture levels observed in this study except 11ºC at 52% soil moisture. This observation paired with the dip in abundance under all soil conditions (except 24ºC with 23% moisture) after adding additional water 15 DAI indicate *T. atroviride* CHS 861 is sensitive to excess moisture like most fungi due to osmotic pressure and limited access to oxygen (Morris and Blackwood, 2007).

Trichoderma atroviride in non-sterile soil

While abundance in the sterile soil elucidated the potential growth of *T. atroviride* CHS 861, these observations excluded the effects of adding the *T. atroviride* strain to a non-sterile, competitive soil microbial community. In the nonsterile soil, mean *T. atroviride* abundance was highest in the samples incubating at 11ºC with a soil moisture range of 23% (Table 4.1). Samples incubating at 32ºC with a soil moisture range of 23% exhibited the lowest mean abundance. The ANCOVA model yielded similar results to those used for the sterile samples; abundance score means significantly differed among temperature levels, between soil moisture conditions, and within the interaction between soil temperature and moisture (Table 4.2). Mean abundance was significantly higher at 11ºC compared to 32ºC soil moisture and higher at 23% soil moisture than 51%. The native microbial community in the soil regulated *T. atroviride* proliferation as indicated by lower mean abundances in the nonsterile soil compared to the sterile soil (Tables 4.3 and 4.4) and the fluctuation in abundance across incubation time under all soil conditions except at 11ºC in 23% soil moisture (Figure 4.2).

The ratio between *T. atroviride* and unidentified fungi abundance signified the degree to which *T. atroviride* was competitive in the native soil microbial community. Under soil conditions of 11ºC in 23% moisture, *T. atroviride* thrived among the native soil microbial community which point to the enhanced competition of the fungus at cooler temperatures with consistently higher ratios of mean abundance of *T. atroviride* to unidentified fungi throughout the incubation period (Tables 4.4). The ANCOVA model confirmed temperature was influential on the competitive nature of *T. atroviride*, while soil moisture was not (Table 4.5). The ratio between *T. atroviride* and unidentified fungi abundance was significantly higher at 11ºC than 24ºC and 32ºC. These observations align with previous knowledge regarding the origin of *T.*

atroviride CHS 861 from sub-arctic soils and performing as an effective biocontrol agent for diseases like *Microdochium nivale* var. *majus* (Wollen W.) and *Typhula* spp. (Pers.) Fr. commonly known as pink or white snow mold respectively (Wong and McBeath, 1999).

T. atroviride was also competitive throughout the duration of the trial at 24ºC and in 23% soil moisture levels as indicated by its abundance rebounding from the increased moisture levels added 15 DAI and demonstrating its survivability among a range of temperatures (Table 4.6). *T. atroviride* did not survive through the 57-d experiment under the other temperature and soil moisture conditions. By comparing the death of *T. atroviride* populations in those nonsterile soil samples to the sterile soil under the same conditions, one possible cause may be attributed to the proliferation of osmophilic or thermophilic microorganisms that outcompeted *T. atroviride* (Morris and Blackwood, 2007).

Temperature and soil moisture effects on Trichoderma atroviride growth rate

The Kruskal Wallis rank-sum tests in both sterile and non-sterile soils did not identify temperature as having a significant effect on growth rate, i.e., *T. atroviride* growth rates did not significantly correlate with temperature in both sterile and nonsterile soils as initially predicted (Table 4.7). *T. atroviride* CHS 861 appears to be comparably viable at 11ºC to 32ºC, a subset of the temperature range previously described by Wong and McBeath (1999).

Negative correlations between *T. atroviride* growth rate and soil moisture were also observed in sterile and nonsterile soils as predicted (Table 4.8), although the relationship was significant only in the sterile soil (Table 4.7). Total fungal growth rates in non-sterile soils also exhibited significant responses to soil moisture conditions ($p=0.0034$, $\alpha=0.05$); average growth rate was 2.7×10^{-2} k d^{-1} and -4.8×10^{-3} k d^{-1} in moisture levels of 23% and 51% respectively. By the end of the trial, *T. atroviride* populations were not detected in any soils under moisture

conditions of 51% (Table 4.4). These results emphasize overall fungal sensitivity to wet soil conditions typically caused by irrigation or rainfall events.

While not statistically evaluated, one notable observation was the comparison between sterile and non-sterile soils at 32ºC in 23% moisture conditions (Table 4.8). The collapse of *T. atroviride* populations in the non-sterile soil compared to the survival of the fungus in the sterile soil suggested that the native microbial community attributed to the demise of *T. atroviride* at 32ºC with soil moisture levels of 23%. Both abundance and growth rate were highest for *T. atroviride* in the absence of microbial communities at 11ºC in 23% soil moisture level, but the decline in growth rate indicated a possible mediation effect from competitors on *T. atroviride* proliferation from the native soil community (Morris and Blackwood, 2007).

Trichoderma atroviride in Georgia soils

The implications of the data collected in this study demonstrated that *T. atroviride* CHS 861 is the most biologically competitive in simulated soil conditions of 11ºC and 24ºC with soil moisture levels ranging from 23. *T. atroviride* CHS 861 may be the most competitive, and thus most efficacious during early to late spring in Georgia. *T. atroviride* CHS 861 is sensitive to wet conditions like most fungi due to limited access to oxygen (Morris and Blackwood, 2007). The study suggested that increased moisture hinders the survival of established *T. atroviride* CHS 861 in soil, making active populations particularly susceptible to irrigation and rainfall events.

The phenomena among the abundance and growth rates among various combinations of soil temperature and moisture of *T. atroviride* CHS 861 observed in this study will not be universal among other Georgia soils. Soil microbial communities, soil types, and environmental factors vary widely throughout the state and *T. atroviride* CHS 861 is apt to behave with varying degrees of efficacy in response to a complex set of parameters not examined in this research.

CONCLUSIONS

T. atroviride CHS 861 growth rates were comparable among temperatures, with no significant correlation between the two parameters in both sterile and non-sterile soils. Soil moisture had a significant negative correlation with growth rate in nonsterile samples, while the relationship between growth rate in sterile and nonsterile soil suggested a possibility of moisture influencing the growth of competitors to *T. atroviride* CHS 861 native to the soil microbial community. Average abundance of *T. atroviride* CHS 861 over the 57-d incubation period was the highest under soil conditions of 24ºC and moisture range of 23% in the sterile soil, indicating that these conditions may be optimal for *T. atroviride* CHS 861 alone. However, *T. atroviride* CHS 861 will inevitably be applied to existing soil communities, so results from the nonsterile soil provide practical implications. The fungal inoculant displayed its most competitive nature at 11ºC and 24ºC at a soil moisture level of 23% in the non-sterile soil samples, indicating that the fungal strain may most efficacious as disease control agents when applied in early spring to late spring in Georgia.

REFERENCES

Aamlid, T.S., G. Thorvaldsson, F. Enger, and T. Pettersen. 2012. Turfgrass species and varieties for integrated pest management of Scandinavian putting greens. Acta Agric. Scand., Sect. B. 62:10–23.

Benítez, T., A.M. Rincón, M.C. Limón, A.C. Codón. 2018. Biocontrol mechanisms of *Trichoderma* strains. Int. Microbiol. 7:249-260.

Boulter, J.J., G.J. Boland, and J.T. Trevors. 2000. Compost: A study of the development process and end-product potential for suppression of turfgrass disease. World J. Microb. Biot. 16:115–134.

Christians, N.E., A.J. Patton, and Q.D. Law. 2017. Fundamentals of turfgrass management. 5th ed. John Wiley & Sons, Hoboken, NJ.

Daryaei, A., E.E. Jones, H. Ghazalibiglar, T.R. Glare, and R.E. Falloon. 2016a. Culturing conditions affect biological control activity of *Trichoderma atroviride* against *Rhizoctonia solani* in ryegrass. Journal of Applied Microbiology. 121:461-472. doi:10.1111/jam.13163

Daryaei, A., E.E. Jones, H. Ghazalibiglar, T.R. Glare, and R.E. Falloon. 2016b. Effects of temperature, light and incubation period on production, germination and bioactivity of *Trichoderma atroviride*. Journal of Applied Microbiology. 120:999-1009.

Daryaei, A., E.E. Jones, T.R. Glare, and R.E. Falloon. 2016c. Nutrient amendments affect *Trichoderma atroviride* conidium production, germination and bioactivity. Biological Control. 93:8-14. doi:10.1016/j.biocontrol.2015.11.003

Daryaei, A., E.E. Jones, T.R. Glare, and R.E. Falloon. 2016d. pH and water activity in culture media affect biological control activity of *Trichoderma atroviride* against *Rhizoctonia solani*. Biological Control. 92:24-30. doi:10.1016/j.biocontrol.2015.09.001

Elliott, M., S.F. Shamoun, G. Sumampong, D. James, S. Masri and A. Varga. 2009. Evaluation of several commercial biocontrol products on European and North American populations of *Phytophthora ramorum*. Biocontrol Sci. Techol. 19:1007-1021, doi:10.1080/09583150903243870

Gómez-Rodríguez, E.Y., E.E. Uresti-Rivera, O.A. Patrón-Soberano, M. A. Islas-Osuna, A. Flores-Martínez, L. Riego-Ruiz, M.T. Rosales-Saavedra, and S. Casas-Flores. 2018. Histone acetyltransferase TGF-1 regulates *Trichoderma atroviride* secondary metabolism and mycoparasitism. PLOS One 13(5):1-23. doi:10.1371/journal.pone.0193872

Harman, G.E. 2006. Overview mechanisms and uses of *Trichoderma* spp. Phytopathology. 96:190-194. doi:10.1094/PHYTO-96-0190

Harman, G.E., C.K. Hayes, and K.L. Ondik. 1998. Asexual genetics in *Trichoderma* and *Gliocladium*: Mechanisms and implications. In: Kubicek, C.P. and G.E. Harman, editors, *Trichoderma* and *Gliocladium*. Volume 1: Biology, taxonomy, and genetics. Taylor & Francis, Bristol, PA. p. 243-270.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet, and M. Lorito. 2004. *Trichoderma* species—Opportunistic, avirulant plant symbionts. Nat. Rev. 2:43–56. doi:10.1038/nrmicro797

Kiriga, A.W., S. Haukeland, G.M. Kariuki, D.L. Coyne, and N.V. Beek. 2018. Effect of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial pineapple production in Kenya. Biol. Control 119:27-32. doi.org/10.1016/j.biocontrol.2018.01.005

Klein, D., and D.E. Eveleigh. 1998. Ecology of *Trichoderma*. Kubicek, C.P. and G.E. Harman, editors, *Trichoderma* and *Gliocladium*. In: Volume 1: Biology, taxonomy, and genetics. Taylor & Francis, Bristol, PA. p. 57-74.

Kowalska, B., U. Smolińska, M. Szczech, and J. Winciorek. 2017. Application of organic waste material overgrown with *Trichoderma atroviride* as a control strategy for *Sclerotinia sclerotiorum* and *Chalara thielavioides* in soil. J. Plant Prot. Res. 57:205-211. doi:10.1515/jppr-2017-0027

Martinez, A., M. Pearce, and L. Burpee. 2017. Turfgrass diseases in Georgia: Identification and control. Univ. Ga. Ext.

https://secure.caes.uga.edu/extension/publications/files/pdf/B%201233_7.PDF (accessed 14 May 2018).

Morris, S.J., and C.B. Blackwood. 2007. The soil habitat. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 195-229.

Nawrocka, J., M. Szczech, U. Małolepsza. 2018. *Trichoderma atroviride* enhances phenolic synthesis and cucumber protection against Rhizoctonia solani. Plant Protect. Sci. 54:17-23. doi.org/10.17221/126/2016-PPS

Nelson, E.B. 1997. Microbial inoculants for the control of turfgrass diseases. Int. Turfgrass Soc. Res. J. 8:791–811.

Schimel, J. 2007. Soil microbiology, ecology, and biochemistry for the 21st century. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 503-514.

Siddiquee, S. 2017. Morphology-based characterization of *Trichoderma* species. In: V.K. Gupta and M.G. Tuohy, editors, Practical handbook of the biology and molecular diversity of *Trichoderma* species from tropical regions. Fungal Biology Ser. Springer Nature, Cham, Switzerland. p. 41-73. doi:10.1007/978-3-319-64946-7

Verma, M., S.K. Brar, R.D. Tyagi, R.Y. Surampalli, and J.R. Valéro. 2007. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochemical Engineering Journal. 37:1-20. doi:10.1016/j.bej.2007.05.012

Voroney, R.P. 2007. The soil habitat. In: E. A. Paul, editor, Soil microbiology, ecology, and biochemistry. 3rd ed. Academic Press, Boston, MA. p. 25-49.

Wong, P.T.W., and J.H. McBeath. 1999. Plant protection by cold-adapted fungi. In: R. Margesin, and F. Schinner, editors, Biotechnological applications of cold-adapted organisms. Springer, Berlin, Heidelberg. p.177-190.

Table 4.1. Summary statistics of *T. atroviride* abundance (CFU g^{-1}) in inoculated sterile and nonsterile soils over a 57-d incubation period pooled by temperature (11ºC, 24ºC, and 32ºC) and soil moisture (23% and 51%).

			T. atroviride abundance (CFU g^{-1})				
Soil	Temperature (ºC)	Soil moisture (%)	Mean	±SD	Median	Min	Max
Sterile	11	23	1.5×10^{5}	1.2×10^{5}	1.3×10^{5}	1.3×10^{3}	3.8×10^{5}
	24		6.0×10^{5}	4.4×10^{5}	4.9×10^{5}	1.4×10^{5}	1.6×10^{6}
	32		3.8×10^{5}	2.1×10^{5}	3.4×10^{5}	1.1×10^{5}	8.9×10^{5}
	11	51	3.1×10^{3}	7.9×10^{3}	0	0	3.4×10^{4}
	24		1.1×10^{5}	6.2×10^{4}	9.4×10^{4}	2.2×10^{4}	2.8×10^{5}
	32		1.6×10^{5}	9.5×10^{4}	1.4×10^{5}	4.9×10^{4}	3.7×10^{5}
Nonsterile	11	23	1.6×10^{4}	9.9×10^{3}	1.7×10^{4}	0	4.2×10^{4}
	24		2.4×10^{3}	5.6×10^{3}	1.3×10^{2}	0	2.3×10^{4}
	32		1.4×10^{2}	3.4×10^{2}	0	0	1.3×10^{3}
	11	51	3.3×10^{2}	6.0×10^{2}	0	0	2.0×10^{3}
	24		1.6×10^{2}	4.7×10^{2}	0	0	2.0×10^{3}
	32		1.3×10^{3}	3.0×10^{3}	0	0	1.0×10^{4}

Table 4.2. ANCOVA of *T. atroviride* abundance (CFU g^{-1}) in sterile and nonsterile soils over a 57-d incubation period pooled by temperature (11ºC, 24ºC, and 32ºC) and soil moisture (23% and 51%).

	Main effect *p*-value (α=0.05)	
Parameter	Sterile soil	Non-sterile soil
R^2*	0.803	0.621
Temperature	<0.0001	0.0042
Soil moisture	<0.0001	0.0029
Temperature × soil moisture	0.0037	0.0320
DAI	0.1516	0.0451

* = R^2 values indicate the fit of the overall model describing response data.

Table 4.3. Mean *T. atroviride* abundance (CFU g^{-1}) in sterile and nonsterile soils over a 57-d incubation period pooled by temperature (11ºC, 24ºC, and 32ºC) with 23% soil moisture.

		Mean ± SD *T. atroviride* abundance (CFU g^{-1})				
Soil	Temperature (ºC)	5 DAI	12 DAI	19 DAI	26 DAI	57 DAI
Sterile	11	$2.5\times10^{3} \pm 1.0\times10^{3}$	$9.6\times10^{4} \pm 1.5\times10^{4}$	$1.4\times10^{5} \pm 2.9\times10^{4}$	$3.0\times10^{5} \pm 5.6\times10^{4}$	$2.2\times10^{5} \pm 1.1\times10^{5}$
	24	$1.6\times10^{5} \pm 2.0\times10^{4}$	$3.8\times10^{5} \pm 1.4\times10^{5}$	$5.1\times10^{5} \pm 5.4\times10^{4}$	$5.6\times10^{5} \pm 1.7\times10^{5}$	$1.4\times10^{6} \pm 2.1\times10^{5}$
	32	$1.2\times10^{5} \pm 2.3\times10^{4}$	$4.1\times10^{5} \pm 1.1\times10^{5}$	$2.6\times10^{5} \pm 2.9\times10^{4}$	$5.3\times10^{5} \pm 6.7\times10^{4}$	$5.9\times10^{5} \pm 2.4\times10^{5}$
Nonsterile	11	$2.8\times10^{3} \pm 2.2\times10^{3}$	$1.5\times10^{4} \pm 2.8\times10^{3}$	$1.4\times10^{4} \pm 7.0\times10^{3}$	$2.1\times10^{4} \pm 5.7\times10^{3}$	$2.5\times10^{4} \pm 1.1\times10^{4}$
	24	$9.4\times10^{2} \pm 1.9\times10^{3}$	$3.3\times10^{2} \pm 2.9\times10^{2}$	$5.7\times10^{3} \pm 1.1\times10^{4}$	$2.2\times10^{3} \pm 4.1\times10^{3}$	$2.3\times10^{3} \pm 4.2\times10^{3}$
	32	0 ± 0	$3.1\times10^{2} \pm 6.3\times10^{2}$	$4.0\times10^{2} \pm 3.8\times10^{2}$	0 ± 0	0 ± 0

Table 4.4. Mean *T. atroviride* abundance (CFU g^{-1}) in sterile and nonsterile soils over a 57-d incubation period pooled by temperature (11ºC, 24ºC, and 32ºC) with 51% soil moisture.

		Mean ± SD *T. atroviride* abundance (CFU g^{-1})				
Soil	Temperature (ºC)	5 DAI	12 DAI	19 DAI	26 DAI	57 DAI
Sterile	11	$1.2\times10^{4} \pm 1.5\times10^{4}$	$5.0\times10^{1} \pm 1.0\times10^{2}$	$3.7\times10^{2} \pm 7.5\times10^{2}$	$1.1\times10^{2} \pm 1.3\times10^{2}$	$2.9\times10^{3} \pm 2.8\times10^{3}$
	24	$8.4\times10^{4} \pm 4.8\times10^{4}$	$1.3\times10^{5} \pm 4.7\times10^{4}$	$1.1\times10^{5} \pm 5.5\times10^{4}$	$1.5\times10^{5} \pm 1.1\times10^{5}$	$7.0\times10^{4} \pm 1.7\times10^{4}$
	32	$2.2\times10^{5} \pm 6.7\times10^{4}$	$2.1\times10^{5} \pm 1.0\times10^{5}$	$1.4\times10^{5} \pm 1.6\times10^{5}$	$1.1\times10^{5} \pm 2.7\times10^{4}$	$1.2\times10^{5} \pm 4.2\times10^{4}$
Nonsterile	11	$1.0\times10^{3} \pm 1.2\times10^{3}$	$3.0\times10^{2} \pm 1.2\times10^{2}$	$2.7\times10^{2} \pm 3.2\times10^{2}$	$1.1\times10^{2} \pm 2.1\times10^{2}$	0 ± 0
	24	0 ± 0	$7.5\times10^{2} \pm 9.0\times10^{2}$	$5.2\times10^{1} \pm 1.0\times10^{2}$	0 ± 0	0 ± 0
	32	$5.0\times10^{2} \pm 1.0\times10^{3}$	$6.0\times10^{3} \pm 4.3\times10^{3}$	0 ± 0	0 ± 0	0 ± 0

Table 4.5. ANCOVA of *T. atroviride* to unidentified fungi abundance ratio in sterile and nonsterile soils over a 57-d incubation period pooled by temperature (11ºC, 24ºC, and 32ºC) and soil moisture (23% and 51%).

	Main effect *p*-value (α=0.05)
Parameter	Sterile soil
R^2*	0.591
Temperature	0.0013
Soil moisture	0.5778
Temperature × soil moisture	0.0668
DAI	0.0068

* = R^2 values indicate the fit of the overall model describing response data.

Table 4.6. Mean ratio of *T. atroviride* to unidentified fungi abundance (%) in nonsterile soil over a 57-d incubation period pooled by temperature (11ºC, 24ºC, and 32ºC) and soil moisture (23% and 51%).

Soil moisture (%)	Temperature (ºC)	Mean ± SD *T. atroviride* to unidentified fungi (%)				
		5 DAI	12 DAI	19 DAI	26 DAI	57 DAI
23	11	75 ± 74	70 ±11	19 ± 7.5	19 ± 4.9	20 ± 7.1
	24	4.2 ± 8.3	2.4 ± 2.1	0 ± 0	2.0 ± 0.94	0.59 ± 0.83
	32	0 ± 0	1.9 ± 3.8	2.8 ± 2.2	0 ± 0	0 ± 0
52	11	33 ± 47	11 ± 4.1	18 ± 21	1.9 ± 3.7	0 ± 0
	24	0 ± 0	38 ± 59	0.69 ± 1.4	0 ± 0	0 ± 0
	32	2.2 ± 4.9	138 ± 88	0 ± 0	0 ± 0	0 ± 0

Table 4.7. Kruskal-Wallis rank-sum tests of mean growth rate (k d^{-1}) of *T. atroviride* in sterile and nonsterile soils observed in nonsterile soil over a 57-d incubation period at three temperature levels (11ºC, 24ºC, and 32ºC) and two levels of soil moisture (23% and 51%).

	Temperature			Soil moisture		
Parameter	Level	Sterile soil	Nonsterile soil	Level	Sterile soil	Nonsterile soil
χ^2		1.37	0.100		12.0	3.83
p-value*		0.5054	0.9511		0.0005	0.0503
Mean abundance score	11ºC	12.4	13.1	23%	17.5	15.3
	24ºC	14.6	12.3	51%	7.50	9.75
	32ºC	10.5	12.1			

*Significant *p*-values indicate differences among distributions of mean score abundance across temperature or soil moisture levels ($p<\chi^2$; $\alpha=0.05$).

Table 4.8. Mean *T. atroviride* growth rate (k d^{-1}) in inoculated sterile and nonsterile soil samples over a 57-d incubation period pooled by temperature (11ºC, 24ºC, and 32ºC) and soil moisture (23% and 51%).

		Mean ± SD *T. atroviride* growth rate (k d^{-1})	
		Soil moisture	
Soil	Temperature (ºC)	23%	51%
Sterile	11	$1.5\times10^{-2} \pm 6.4\times10^{-3}$	$-8.4\times10^{-1} \pm 1.3\times10^{0}$
	24	$3.0\times10^{-2} \pm 6.1\times10^{-3}$	$-1.8\times10^{-2} \pm 3.2\times10^{-2}$
	32	$1.5\times10^{-2} \pm 6.7\times10^{-3}$	$-1.8\times10^{-2} \pm 3.0\times10^{-3}$
Nonsterile	11	$1.7\times10^{-2} \pm 6.5\times10^{-3}$	$-1.4\times10^{-1} \pm 1.6\times10^{-1}$
	24	$7.7\times10^{-3} \pm 4.3\times10^{-2}$	$-6.9\times10^{-3} \pm 1.4\times10^{-2}$
	32	$-7.9\times10^{-3} \pm 1.6\times10^{-2}$	$-1.2\times10^{-2} \pm 2.4\times10^{-2}$

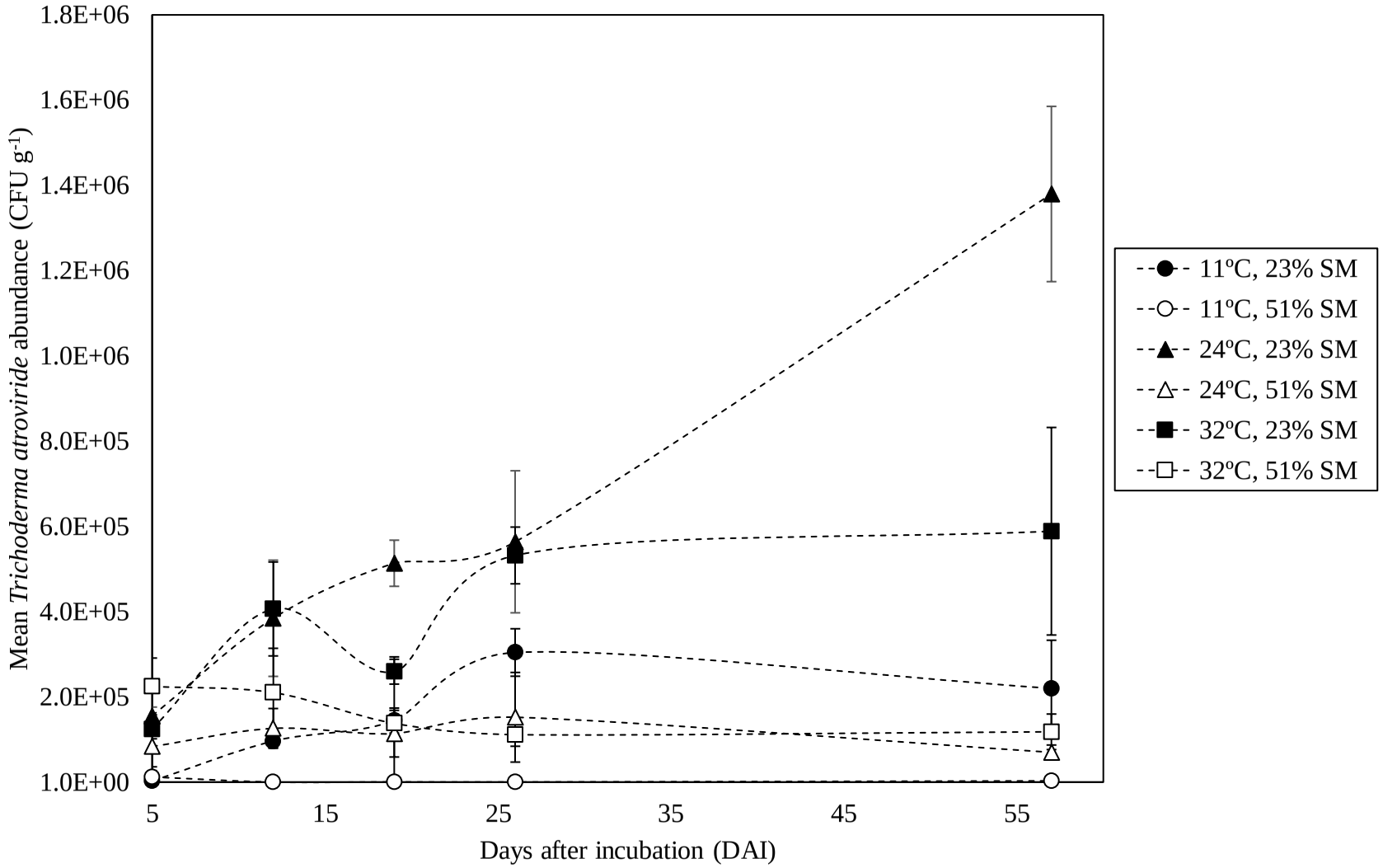

Figure 4.1. Mean ± SD *T. atroviride* abundance (CFU g^{-1}) in sterile soil samples over a 57-d incubation period at three temperatures (11ºC, 24ºC, and 32ºC) and two levels of soil moisture (23% and 51%).

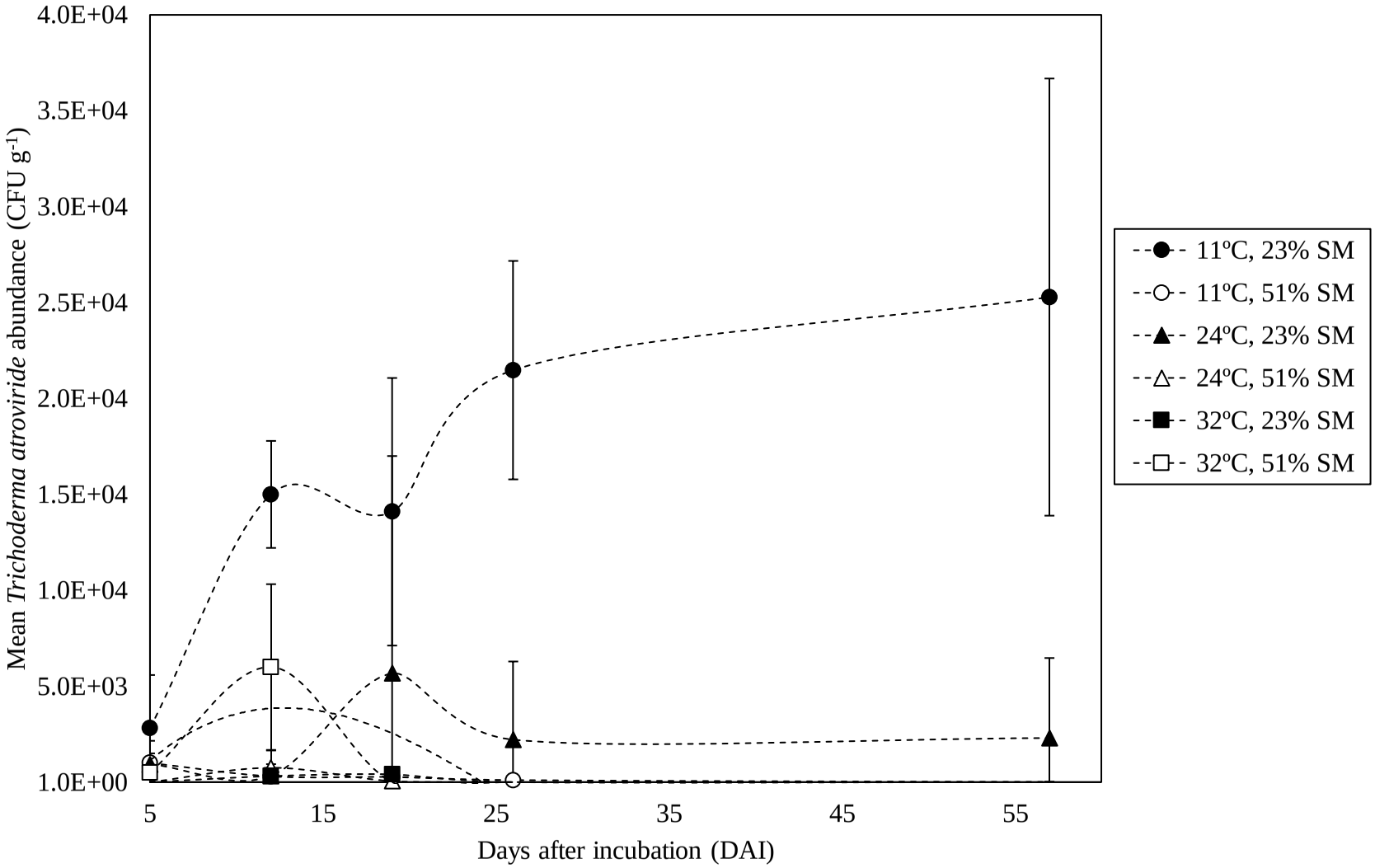

Figure 4.2. Mean ± SD *T. atroviride* abundance (CFU g^{-1}) in nonsterile soil samples over a 57-d incubation period at three temperatures (11ºC, 24ºC, and 32ºC) and two levels of soil moisture (23% and 51%).

CHAPTER 5

SUMMARY AND CONCLUSIONS

This research elucidated various short-term responses of native soil microbial communities to some elements of a turf management program in a Georgia golf course. Microbial abundance determined by qPCR assays confirmed the predominance of bacteria. Total bacteria, including AOB, exhibited sensitivity to wetting agents (C+D and Rev) and *Trichoderma atroviride* CHS 861 in PH treatments, while fungi and AOA did not respond to such treatments in the putting green. However, seasonal changes from late spring to early summer influenced the decline of unidentified fungi and stabilization in AOA and AOB populations in the putting green over the 84-d trial period. In the fairway, total bacteria exhibited growth in average population size in late June after two PGR applications of PM possibly in response to increased root development and mature turfgrass residue. Neither PGR treatments (PM and CL) affected fungi, AOA, and AOB populations. The increase in mineralized SOM and/or fertilization during the growing season likely attributed to higher AOB abundance observed in the fairway over time.

Phosphatase, urease, and soil respiration lab assays were used to evaluate microbial functions in the golf course soils. Phosphatase activity was not affected by any treatment applications in the putting green and fairway soils, indicating that P cycling may not be impacted by the products tested in the field. However, urease activity response indicated that the wetting treatment C+D and PGR treatment CL affected N cycling in the putting green and fairway respectively. The higher rates of urease observed after C+D applications are indicative of

stimulated ureolytic microbial activity and/or the release of soil colloid-complexed ureases into the soil solution by the wetting agent in the putting green. In the fairway, depressed urease activity observed after CL treatments. Higher rates of phosphatase and urease activity occurred in the fairway compared to the putting green, which correlates to less disturbance, higher levels of organic material, and a higher clay fraction in the fairway. In both putting green and fairway soils, phosphatase and urease activity were more pronounced in top 5 cm depth than the 5 to 10 cm depth due to decreasing substrate availability down the soil profile. Soil respiration indicated immediate, short-term microbial responses to Rev and PH treatments in the putting green and PM and CL treatments in the fairway. The stabilization in soil respiration levels between 6 to 8 wks after treatments in the fairway and comparable respiration levels between the start and end of the putting green trial in all treatments suggest that the routine microbial activity in both soils are resilient to golf course management practices.

The laboratory study provided insight into the survivability of *T. atroviride* CHS 861 among a soil microbial community native to a Georgia sandy loam soil. Growth rates were comparable among three temperatures (11ºC, 24ºC, and 32ºC) in sterile and non-sterile soils. Increasing soil moisture from 23 to 51% hindered the survivability of established *T. atroviride* CHS 861 in soil, which indicates active populations might particularly be susceptible to irrigation and rainfall events. The influence of moisture has also influenced the growth of competitors to *T. atroviride* CHS 861 native to the microbial community in the soil. *T. atroviride* CHS 861 was the most competitive at11ºC and 24ºC at a soil moisture level of 23%, although the inoculant demonstrated an ability to survive in a temperature range of 11ºC and 32ºC when uninhibited by other soil biota.

The practical implications of this research provide some insight into the dynamics of common turf care treatments, soil microbial communities, and the ecosystem services they provide to turfgrasses. The findings of this work are intended to support consumers and professionals in the turf industry as they seek out products to establish sustainable turfgrass systems. Finally, this research is intended to provide additional research opportunities to examine long-term impacts of turf care products on soil microbial communities, evaluate the greater implications of management programs on multiple trophic levels in a turfgrass ecosystem, and conducting similar evaluations in other kinds of turfgrass systems.