IMPACTS OF TURF CARE PRODUCTS ON SOIL BIOLOGICAL HEALTH

by

WINNIEFRED GRIFFIN

(Under the Direction of Mussie Y. Habteselassie)

ABSTRACT

Research was conducted on golf courses in Johns Creek, Milton, and Griffin GA from May 2018 to October 2019 to evaluate long-term effects of wetting agents, plant growth regulators, and biological products on microbial abundance and function using quantitative polymerase chain reaction, phosphatase, urease, and soil respiration assays. In the putting green trial Revolution improved phosphatase activity and whereas Cutless enhanced phosphatase activity in the fairway. In the fairway, Cutless and Primo Maxx depressed urease activity over time as compared with the Control and Trimmit. The biologicals overall did not show much significant differences between the two trials as compared to the control with the exception of BP2 applied subsurface resulting in enhanced soil respiration, urease activity, and ammonia oxidizers. However, it is important to note that this is a relatively short study time. A long-term study might provide a better insight in the future.

INDEX WORDS: Wetting agent, plant growth regulator, biological products, phosphatase activity, urease activity, soil respiration, microbial abundance, quantitative

polymerase chain reaction, disease suppressive nature, turf quality.

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BS, University of Georgia, 2017

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

2020

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December 2020

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ACKNOWLEDGEMENTS

I would like to thank Dr. Mussie Habteselassie, my advisor, for his support and patience 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 continued encouragement and guidance.

Thank you to Mark Hoban, the superintendent of Rivermont Golf Club and Todd Lime, the superintendent of Echelon Golf Club, for providing the space, time, and tools to support the field components of this research. I would also like to extend my gratitude to Dr. Alfredo Martinez and Brian Vermeer for their guidance, knowledge and immense help with the inoculation study and much more.

I want to extend my gratitude to Hunter Donahue, Miranda Barr, Bright Ofori, Camilla Drocco, and countless others for their tremendous help throughout the duration of my graduate program. You all have provided me the helping hands and much needed mental support to make it through to the end.

Finally, I want to acknowledge my father, David Griffin, for his continued support and encouragement. I truly couldn't have done this without you dad.

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Chapter One

INTRODUCTION

Turfgrasses have been utilized for a myriad of landscapes, covering nearly 50 million acres and providing functional, recreational, and aesthetic benefits for humans for many centuries. (Beard 1994; Falk 1976; Milesi et al. 2005). These benefits make turfgrasses an important social, environmental, and economic resource (Strandberg et al., 2012). In turfgrass for golf courses, superintendents are often under pressure to maintain high quality turf under climatic, pest and use-induced stresses (Rossi, 2005; Duncan and Carrow, 1999). This demand requires extensive use of inputs that include fertilizers, pesticides, herbicides, wetting agents, water, plant growth regulators. This makes the sector among the most expensive sectors in agriculture. Reducing inputs is therefore important for the future of the golf course industry.

Reducing input is also important from the point of view of reducing the environmental footprint of golf courses. The demand for greater environmental protection coupled with increasing demand on natural resources has pushed many superintendents to reevaluate course management. In order to move towards a more sustainable management, superintendents need to begin focusing on resource-use efficiency, reducing costs, and minimizing environmental impacts (Strandberg et al., 2012). However, the impact of some of these inputs in the environment is not clear.

Among the most commonly used products in turfgrass management are wetting agents and plant growth regulators. In a survey of 600 superintendents conducted by Karnok et al.

(2004), 87% of them reported using wetting agents as part of their regular maintenance program. Wetting agents are used to address the problem of localized dry spots (LDS) prevalent in turfgrass soils during the summer caused by soil water repellency (Karnok 2004; Karnok and Tucker, 2001). The occurrence of LDS causes water stress and negatively affects turf quality. Wetting agents are alcohol-based surfactants that change the surface properties of the sand to decrease the occurrence of LDS. Their impact on soil microbial community is largely unknown. Plant growth regulators, on the other hand, are marketed to promote healthier turf with the ability to withstand various types of stresses. Growth regulators are designed to slow down production of hormones (e.g., gibberellic acid) and thereby to minimize vertical shoot growth while promoting lateral and below-ground root growth. There are several studies that tested their efficacy on turfgrass growth and quality with mixed results (McCann and Huang, 2007; Gardner and Wherley, 2005) but their impact on the turfgrass soil microbial communities has not been examined.

Cost and environmental concerns on use of inputs for maintaining turfgrass have led to the proliferation of biological products that are collectively called biostimulants. These products contain microorganisms (bugs in a jug) and/or organic products that are often marketed as being more sustainable and cheaper alternatives to current products that are commonly used in the golf course industry. This assumes that the biological products are better in stimulating the indigenous soil microorganisms that provide beneficial services. However, there is lack of research in evaluating how effective biological products are, and how they affect the health of the turfgrass system and turf quality.

Research is needed to understand how wetting agents, plant growth regulators, and biologicals affect the soil microbial communities, which play a central role in the

establishment and maintenance of a healthy and thriving turfgrass ecosystem. Decomposition of organic matter is a central role microorganisms play (Myrold and Bottomley, 2008). This process releases nutrients from organic forms to inorganic forms that can be used by the turf and controls the excessive accumulation of thatch. Microorganisms also contribute to the nutrient content of the turfgrass soil through nitrogen fixation. Free-living nitrogen fixers in a turfgrass system can contribute as much as 20 kg N per ha (Boddey and Victoria, 1986). Microorganisms have also been shown to improve phosphorous availability by modifying the pH at microsite levels to solubilize phosphorous containing minerals (Richardson and Simpson, 2011). The symbiotic relationship between fungi and turfgrass roots (mycorrhiza) is widely credited to improving acquisition of nutrients and (Hartnett and Wilson, 2002; Charest and Dalpe, 1997). The role of microorganisms in disease suppression is well documented too (Kerry, 2000). It is therefore logical to ask this: can we enhance the beneficial roles of soil microorganisms to decrease external inputs and maintain a healthy and sustainable turfgrass system? Can biological products play a role in achieving this goal? This requires proper understanding of the impact wetting agents, plant growth regulators and biological products on the health of the soil biology and turf quality.

Significance of the study

The key research question we want to address in this study is this: do wetting agents and plant growth regulators negatively impact the turfgrass soil microbial communities? If so, what aspects (abundance and/or function) of the microbial communities are affected and what are the implications? It is important to address these research questions as they will determine the role of the microbial communities in organic matter decomposition, nutrient

availability, nutrient acquisition and disease suppression in turfgrass systems. If any of these functions are negatively impacted, it will have financial consequences to the superintendents as it will necessitate the use of additional inputs (in the form of commercial products) to make up for the lost benefits. It will also negatively impact the sustainability of the turfgrass ecosystem. It is well documented that less diverse ecosystems are highly susceptible to stresses and require higher levels of maintenance (Brussaard et al. 2007). As such, our goal is to evaluate the impact of selected wetting agents, plant growth regulators, and biological products on the abundance and activity of microorganisms in the field. Secondly, we want to examine the impact of biological products on turf quality and soil health, and if biological products can potentially be supplemental to or more sustainable alternatives to conventional inputs in order to boost the soil health.

REFERENCES

Anderson, T. H., "Microbial Eco-Physiological Indicators to Assess Soil Quality." *Agriculture, Ecosystems and Environment*, vol. 98, no. 1–3, 2003, pp. 285–93, doi:10.1016/S0167-8809(03)00088-4.

Barrios, E. "Soil Biota, Ecosystem Services and Land Productivity." *Ecological Economics*, vol. 64, no. 2, 2007, pp. 269–85, doi:10.1016/j.ecolecon.2007.03.004.

Bartlett, M. D., et al. "Interactions between Microbial Community Structure and the Soil Environment Found on Golf Courses." *Soil Biology and Biochemistry*, vol. 39, no. 7, 2007, pp. 1533–41

Barton, L., and T. D. Colmer. "Ameliorating Water Repellency under Turfgrass of Contrasting Soil Organic Matter Content: Effect of Wetting Agent Formulation and Application Frequency." *Agricultural Water Management*, vol. 99, no. 1, Elsevier B.V., 2011, pp. 1–7, doi:10.1016/j.agwat.2011.08.009.

Beard, J. B., and R. L. Green. "The Role of Turfgrasses in Environmental Protection and Their Benefits to Humans." *Journal of Environmental Quality*, vol. 23, no. 3, 1994, pp. 452–60, doi:10.2134/jeq1994.00472425002300030007x.

Bonanomi, G., et al. "Organic Amendments, Beneficial Microbes, and Soil Microbiota: Toward a Unified Framework for Disease Suppression." *Annual Review of Phytopathology*, vol. 56, no. 1, 2018, pp. 1–20, doi:10.1146/annurev-phyto-080615-100046.

Burns, R. G., et al. "Soil Enzymes in a Changing Environment: Current Knowledge and Future Directions." *Soil Biology and Biochemistry*, vol. 58, Elsevier Ltd, 2013, pp.

216–34, doi:10.1016/j.soilbio.2012.11.009.

Chen, H., et al. “The Soil Microbial Community of Turf: Linear and Nonlinear Changes of Taxa and N-Cycling Gene Abundances over a Century-Long Turf Development.” *FEMS Microbiology Ecology*, vol. 95, no. 2, Oxford University Press, 2019, pp. 1–14, doi:10.1093/femsec/fiy224.

Chen, H., et al. “The Soil Microbial Community of Turf: Linear and Nonlinear Changes of Taxa and N-Cycling Gene Abundances over a Century-Long Turf Development.” *FEMS Microbiology Ecology*, vol. 95, no. 2, Oxford University Press, 2019, pp. 1–14, doi:10.1093/femsec/fiy224.

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

Cotton, S. “Wetting Agents: What Are They and How Do They Work?” *American Scientist*, vol. 106, no. 3, 2018, p. 138, doi:10.1511/2018.106.3.138.

Crecchio, C., et al. *Beneficial Soil Microbiome for Sustainable Agriculture Production*. no. September 2018, pp. 443–81, doi:10.1007/978-3-319-94232-2_9.

Cutler, N. R., et al. “Soil Health and Sustainability.” *Life Sciences*, vol. 62, no. 16, 1998, pp. 1433–41, doi:10.1016/S0024-3205(98)00082-4.

Das, S. K., and A. V., “Soil Enzymology.” *Soil Enzymology*, vol. 22, 2010, pp. 275–85, doi:10.1007/978-3-642-14225-3.

Dell, E. A., et al. “Heat Stress and N Fertilization Affect Soil Microbial and Enzyme Activities in the Creeping Bentgrass (Agrostis Stolonifera L.) Rhizosphere.” *Applied Soil Ecology*, vol. 56, 2012, pp. 19–26, doi:10.1016/j.apsoil.2012.02.002.

Diera, AA, Raymer, PL, Martinez-Espinoza, AD, Bauske, E, Habteselassie,

MY. Evaluating the impact of turf-care products on soil biological health. *J. Environ. Qual*. 2020; 49: 858– 868. https://doi.org/10.1002/jeq2.20080

du Jardin, P. "Plant Biostimulants: Definition, Concept, Main Categories and Regulation." *Scientia Horticulturae*, vol. 196, Elsevier B.V., 2015, pp. 3–14, doi:10.1016/j.scienta.2015.09.021.

Fierer, N., et al. "Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays." *Applied and Environmental Microbiology*, vol. 71, no. 7, 2005, pp. 4117–20, doi:10.1128/AEM.71.7.4117-4120.2005.

Fry, J., et al. *Reducing Fungicide Use on Creeping Bentgrass Greens*. no. July 2006, pp. 77–81.

González-vila, F. J., and A. S. Ball. *Soil Health — a New Challenge for Microbiologists And*. 2013, pp. 13–21.

Harman, G. E., et al. "Non-Target Effects of Fungicide Applications on Microbial Populations of Putting Greens." *USGA Green Sec. Rec.*, vol. 44, no. 4, 2006, pp. 9–12.

Hill, G. T., et al. "Methods for Assessing the Composition and Diversity of Soil Microbial Communities." *Applied Soil Ecology*, vol. 15, no. 1, 2000, pp. 25–36, doi:10.1016/S0929- 1393(00)00069-X.

Kennedy, A. C., and R. I. Papendick. *Microbial Characteristics of Soil Quality*. no. June, 1995, pp. 243–48.

Kokkoris, V., et al. "Site Specificity in Establishment of a Commercial Arbuscular Mycorrhizal Fungal Inoculant." *Science of the Total Environment*, vol. 660, Elsevier B.V., 2019, pp. 1135–43, doi:10.1016/j.scitotenv.2019.01.100.

Lee, J., et al. "Dollar Spot and Brown Patch Incidence in Creeping Bentgrass as Affected by

Acibenzolar-S-Methyl and Biostimulants." *HortScience*, vol. 38, no. 6, 2003, pp. 1223–26, doi:10.21273/hortsci.38.6.1223.

Liu, Y., et al. "Microbial and Soil Properties in Bentgrass Putting Greens: Impacts of Nitrogen Fertilization Rates." *Geoderma*, vol. 162, no. 1–2, Elsevier B.V., 2011, pp. 215–21, doi:10.1016/j.geoderma.2011.02.009.

Lo, C. T., et al. "Improved Biocontrol Efficacy of Trichoderma Harzianum 1295-22 for Foliar Phases of Turf Diseases by Use of Spray Applications." *Plant Disease*, vol. 81, no. 10, 1997, pp. 1132–38, doi:10.1094/PDIS.1997.81.10.1132.

Milesi, C., et al. "Mapping and Modeling the Biogeochemical Cycling of Turf Grasses in the United States." *Environmental Management*, vol. 36, no. 3, 2005, pp. 426–38, doi:10.1007/s00267-004-0316-2.

Moebius-Clune, B.N, et al. *Comprehensive Assessment of Soil Health*. 2016, http://www.nysaes.cornell.edu.

Mueller, S. R., and W. R. Kussow. "Biostimulant Influences on Turfgrass Microbial Communities and Creeping Bentgrass Putting Green Quality." *HortScience*, vol. 40, no. 6, 2005, pp. 1904–10, doi:10.21273/hortsci.40.6.1904.

Mukerji, K. G. "Soil Microbes." *Techniques in Mycorrhizal Studies*, vol. 50, 2002, pp. 7–13, doi:10.1007/978-94-017-3209-3_2.

Mundepi, A., et al. "Ammonia Oxidizers as Biological Health Indicators of Elevated Zn and Cu in Poultry Litter Amended Soil." *Water, Air, and Soil Pollution*, vol. 230, no. 10, Water, Air, & Soil Pollution, 2019, doi:10.1007/s11270-019-4283-x.

Nannipieri, P., et al. "Soil Enzyme Activity: A Brief History and Biochemistry as a Basis for Appropriate Interpretations and Meta-Analysis." *Biology and Fertility of Soils*, vol. 54,

no. 1, Biology and Fertility of Soils, 2018, pp. 11–19, doi:10.1007/s00374-017-1245-6.

Nielsen, M. N., and A. Winding. "Microorganisms as Indicators of Soil Health." *Neri*, vol. NERI Techn, no. 388, 2002, http://www.dmu.dk.

Sahrawat, K. L. (1984). Effects of temperature and moisture on urease activity in semi-arid tropical soils. Plant and Soil, 78(3), 401-408.

Saporta, R., et al. "A Method for a Fast Evaluation of the Biostimulant Potential of Different Natural Extracts for Promoting Growth or Tolerance against Abiotic Stress." *Agronomy*, vol. 9, no. 3, 2019, doi:10.3390/agronomy9030143.

Schlatter, D., et al. "Disease Suppressive Soils: New Insights from the Soil Microbiome." *Phytopathology*, vol. 107, no. 11, 2017, pp. 1284–97, doi:10.1094/PHYTO-03-17-0111- RVW.

Schloter, M., et al. "Indicators for Evaluating Soil Quality." *Agriculture, Ecosystems and Environment*, vol. 98, no. 1–3, 2003, pp. 255–62, doi:10.1016/S0167-8809(03)00085-9.

Sher, Y., et al. "Ammonia Transformations and Abundance of Ammonia Oxidizers in a Clay Soil Underlying a Manure Pond." *FEMS Microbiology Ecology*, vol. 81, no. 1, 2012, pp. 145–55, doi:10.1111/j.1574-6941.2012.01347.x.

Shi, W., et al. "Soil Microbial Community Composition and Function in Turfgrass Ecosystems." *Bioremediation, Biodiversity and Bioavailability*, vol. 1, no. 1, 2007, pp. 72–77.

Shi, W., et al. "Soil Enzyme Activities and Organic Matter Composition in a Turfgrass Chronosequence." *Plant and Soil*, vol. 288, no. 1–2, 2006, pp. 285–96, doi:10.1007/s11104-006-9116-1.

Shi, W., et al. “Soil Microbial Biomass, Activity and Nitrogen Transformations in a Turfgrass Chronosequence.” *Soil Biology and Biochemistry*, vol. 38, no. 2, 2006, pp. 311–19, doi:10.1016/j.soilbio.2005.05.008.

Shimada, M. “Agarose Gel Electrophoresis.” *Tanpakushitsu Kakusan Koso. Protein, Nucleic Acid, Enzyme*, vol. 41, no. 5, 1996, pp. 477–79.

Song, Enzhan, et al. “Influence of Repeated Application of Wetting Agents on Soil Water Repellency and Microbial Community.” *Sustainability (Switzerland)*, vol. 11, no. 16, 2019, pp. 1–13, doi:10.3390/su11164505.

Song, E., et al. “Influence of Repeated Application of Wetting Agents on Soil Water Repellency and Microbial Community.” *Sustainability (Switzerland)*, vol. 11, no. 16, 2019, pp. 1–13, doi:10.3390/su11164505.

Steketee, C. J., et al. “Effects of Genotype and Isolate on Expression of Dollar Spot in Seashore Paspalum.” *HortScience*, vol. 51, no. 1, 2016, pp. 67–73, doi:10.21273/hortsci.51.1.67.

Strandberg, M., et al. “Priorities for Sustainable Turfgrass Management: A Research and Industry Perspective.” *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, vol. 62, no. SUPPL.1, 2012, pp. 3–9, doi:10.1080/09064710.2012.682163.

Taliaferro, C. M. “Diversity and Vulnerability of Bermuda Turfgrass Species.” *Crop Science*, vol. 35, no. 2, 1995, p. 327, doi:10.2135/cropsci1995.0011183x003500020006x.

Thompson, G. L., and J. Kao-Kniffin. “Diversity Enhances NPP, N Retention, and Soil Microbial Diversity in Experimental Urban Grassland Assemblages.” *PLoS ONE*, vol. 11, no. 5, 2016, pp. 1–17, doi:10.1371/journal.pone.0155986.

Thompson, G. L., and J. Kao-Kniffin. “Urban Grassland Management Implications for Soil c and n Dynamics: A Microbial Perspective.” *Frontiers in Ecology and Evolution*, vol. 7, no. AUG, 2019, pp. 1–15, doi:10.3389/fevo.2019.00315.

Trabelsi, D, and R Mhamdi. “Microbial Inoculants and Their Impact on Soil Microbial Communities: A Review.” *BioMed Research International*, vol. 2013, 2013, doi:10.1155/2013/863240.

Trognitz, F., et al. “The Role of Plant-Microbiome Interactions in Weed Establishment and Control.” *FEMS Microbiology Ecology*, vol. 92, no. 10, 2016, pp. 1–15, doi:10.1093/femsec/fiw138.

Utobo, E. B. “Soil Enzymes as Bioindicators of Soil Ecosystem Status.” *Applied Ecology and Environmental Research*, vol. 13, no. 1, 2014, pp. 85–97, doi:10.15666/aeer/1301.

Van Bruggen, A. H. C., and A. M. Semenov. “In Search of Biological Indicators for Soil Health and Disease Suppression.” *Applied Soil Ecology*, vol. 15, no. 1, 2000, pp. 13–24, doi:10.1016/S0929-1393(00)00068-8.

Van Der Heijden, M G. A., et al. “The Unseen Majority: Soil Microbes as Drivers of Plant Diversity and Productivity in Terrestrial Ecosystems.” *Ecology Letters*, vol. 11, no. 3, 2008, pp. 296–310, doi:10.1111/j.1461-0248.2007.01139.x.

Wyngaard, N, et al. “Legacy Effect of Fertilization and Tillage Systems on Nitrogen Mineralization and Microbial Communities.” *Soil Science Society of America Journal*, vol. 80, no. 5, 2016, pp. 1262–71, doi:10.2136/sssaj2016.03.0070.

Yakhin, O. I., et al. “Biostimulants in Plant Science: A Global Perspective.” *Frontiers in Plant Science*, vol. 7, 2017, doi:10.3389/fpls.2016.02049.

Yao, H, et al. “Soil Microbial Community Structure and Diversity in a Turfgrass Chronosequence: Land-Use Change versus Turfgrass Management.” *Applied Soil Ecology*, vol. 34, no. 2–3, 2006, pp. 209–18, doi:10.1016/j.apsoil.2006.01.009.

Zontek, B. Y. Stanley J. *Understanding the Different Wetting Agent Chemistries*. Green Record, vol. 50 2012, pp. 1–6.

CHAPTER 2

LITERATURE REVIEW

Golf course managed soils

Turfgrasses have been used by humans for over ten centuries to enhance environments both aesthetically and functionally. Like all turfgrass systems, golf courses are heavily maintained and cultured by humans. Managed turf accounts for approximately 13,840 mi^2 (35,850 km^2) in the United States (Milesi et al., 2005) and requires three times more water than the United States' most irrigated crop corn (Falk, 1976; Milesi et al., 2005). In 1993, the annual expenditure for maintaining turfgrass in the USA was estimated to be about $25 billion (Cockerham and Gibeault, 1985, Beard 1994) and continues to rise. This high input coupled with high cost makes optimizing management practices key to establishing and maintaining sustainable golf courses. Engineering strong turf with high rooting capability and turnover is a primary goal in golf course maintenance (Beard 1994). The key for future sustainable management of turfgrass will be to increase resource-use efficiency, reduce managing costs and lessen environmental impact by promoting soil health (Bigelow and Soldat, 2013, Strandberg et al., 2012, Beard, 1994).

There is a large drive to preserve natural resources, ecosystem services, and biodiversity. Heavy reliance on fertilizers and pesticides in turfgrass systems subject them to greater national scrutiny to ensure these areas are not contributing to pollution of groundwater (Strandberg et al., 2012). Another goal of sports turf is to maintain high quality surfaces for playability. Irrigation controls growth and nature of the turf while maximizing playability and bounce (Christians et al. 2016, Strandberg et al., 2012, Beard 1994). These and many other methods of golf course management address a variety of

turfgrass stresses to maintain dense, green turfgrass.

Thatch formation in turfgrass soils is also an important management consideration in golf course soils directly related to microbial activity. Thatch is a tightly compressed layer formed between the turf canopy and soil surface as a result of combined root tissues and decomposing organic residues. Thatch is directly produced as a result of soil microorganisms breaking down organic material from dead/dying root tissues and other organisms in various stages of life and decay (Gaussoin et al., 2013). Mature layers of thatch can provide bounce to the soil surface, a habitat for beneficial micro- and macrofauna, and a barrier between chemical inputs and groundwater. However, an overaccumulation of 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 (Christians et al., 2016). Thatch build-up can also be broken up through the process of aerification. Aerating the soil creates openings in the lawn to help air, water and nutrients move into the soil to the grass roots, alleviate soil compaction and help break up thatch (Christians et al., 2016).

Turfgrass can be subjected to many levels of stress dependent on a variety of factors including thatch accumulation, heavy rainfall, drought, heat, and disease (Christians et al., 2016). Turfgrass soils are typically highly disturbed, heavily concentrated, and experience the consequences of soil compaction due to anthropogenic traffic. This reduced pore capacity and altered pore size negatively affect water movement and gas exchange (Bigelow and Soldat, 2013). The combination of soil air and water are key components of the soil matrix that affect both turf growth and microbial activity. Limiting 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).

Golf courses have distinct management intensity levels dependent on differing playing surfaces. Tees and putting greens can be described as anthropogenic soil profiles (Bartlett et al. 2007). Managed ecosystems can contain less diverse microbial communities when compared to its' natural, untouched landscape (Torsvik et al., 2002, Yao et al., 2006). More intensely managed systems including putting greens and tees, which are made up of sandy soils with drainage systems to improve nutrient retention, minimize soil compaction, and produce playable, even turf surfaces (Bigelow and Soldat, 2013; Christians et al., 2016). These spaces tend to receive more frequent applications of fertilizers, wetting agents, and other turf care products due to their inability to retain nutrients as well as fairways (Bartlett et al., 2007; 2009, Christians et al., 2016). Fairways are also managed in a way to accommodate foot traffic and playability; however, these management practices vary widely based on the native soil conditions.

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 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).

Among the most commonly used inputs in golf courses are wetting agents and plant growth regulators. Lately, biological products are extensively marketed to superintendents as alternative products to convention inputs.

Wetting agents: Wetting agents are defined as "any compound that causes a liquid to spread more easily by reducing the surface tension of the liquid" (Zontek and Kostka 2012). Wetting agents are used to address the problem of localized dry spots (LDS) and hydrophobic dry patch prevalent in turfgrass soils during the summer caused by soil water repellency (Karnok 2004; Karnok and Tucker, 2001). Due to shifts in soil use, from blended topsoils to straight sand, the turf is more likely to become hydrophobic (Zontek and Kostak 2012). The occurrence of LDS causes water stress and negatively affects turf quality.

Despite differences among products, several studies have reported wetting agents to be effective in reducing LDS in golf courses (Throssell; 2005; Leinauer et al., 2001; Kostka, 2000). Wetting agents can be useful in rewetting an area which allows for more soil wetting over time leading to prevention of permanent turf loss from wilting (Zontek and Kostka 2012). However, some wetting agents can cause phytotoxicity in turf and require irrigation immediately following application to minimize turf damage (Karnok, 2006). The effect of wetting agents on the turfgrass soil microbial communities is largely unknown. Some studies have reported the inhibition of microbially mediated decomposition of pollutants due to surfactants in non- turfgrass soils, with subsequent changes in microbial populations (e.g.,

Colores et al. 2000; Laha and Luthy, 1991; Song et al. 2019).

Plant Growth Regulators: Plant growth regulators are often marketed to promote healthier turf with the ability to withstand various types of stresses. Growth regulators are designed to slow down production of hormones (e.g., gibberellic acid) and thereby to minimize vertical shoot growth while promoting lateral and below-ground root growth (e.g., PrimoMaxx and Trimmit 2SC, Syngenta; Cutless, SePRO). There are several studies that tested their efficacy on turfgrass growth and quality with mixed results (McCann and Huang, 2007; Gardner and Wherley, 2005) but their impact on the turfgrass soil microbial communities has not been examined. It is important to study whether these products have similar inhibition effect on the microorganisms, and what the implications would for their use in the turfgrass soil system.

Biological Products: The need for more sustainable, environmentally friendly management practices has led to the proliferation of many different biological products also known as plant biostimulants. These products are defined as "any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content" (du Jardin, 2015). These products claim to be beneficial to soil systems by stimulating and enhancing already present microbial communities. Plant biostimulants fall under a variety of categories based on composition, substance, and targeted intent (Calvo et al., 2014; du Jardin, 2012; Halpern et al., 2015). These categories include: humic and fulvic acids, inorganic compounds, beneficial fungi/bacteria, seaweed extracts, and N-containing compounds (du Jardin, 2015). The microbial products often include bacterial and fungal inoculants that are stated to give a wide range of benefits, including stimulation of root formation and growth,

promotion of nitrogen fixation, prevention and control of foliar and root diseases, and increased resistance to biotic and abiotic stresses (Yakhin et al, 2017).

Not only are these products advertised to provide a more lasting environment, they also appeal to superintendents as a means to reduce management inputs. Thus, many biostimulants are categorized by their ability to provide both economic and environment benefits (du Jardin, 2015). While these products are making their way into the market, there is still very little research in the viability and efficacy of these products. Most research conducted has been within the confines of laboratories providing controlled environments for testing. One big concern is how these products will interact in field operations where the environment remains ever changing.

Soil Health in Turfgrass Management

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." Soil is a critically important component in the maintenance of local, regional, and global environmental quality. 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 impacts soil health (Gaussoin et al., 2013). 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. This rhizosphere represents one of the most diverse habitats of

microorganisms and is essential to ecosystem functioning (Trabelsi and Mhamdi 2013).

Soil microorganisms are just one group that can serve as measure of soil health for superintendents to assess management practices and in turn 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 consist of a variety of species that can be very beneficial for turfgrass productivity. Healthy, soils promote the establishment of diverse, conducive species, and the presence of some important species involved in nutrient cycling such as N-fixing bacteria *Rhizobium* spp. (Barrios, 2007; van Bruggen and Semenov, 2000; Fierer et al., 2007; Arias, 2005). These microorganisms can serve as simple indicators of soil quality. Beneficial microorganisms also play a key role in the functioning of ecosystem services, including global cycling of organic matter, nutrient recycling/reworking, 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; Arias, 2005).

Microbial community composition, enzyme activity, and soil respiration serve as some soil health indicators as demonstrated in past research connecting these elements of the soil habitat to turfgrass studies. The resilience of soil is largely linked to its diversity; in that the greater the diversity the greater the resilience (Arias, 2005). Biodiversity in soil systems is best evaluated at the microbial level by group, such as bacteria, fungi and ammonia-oxidizers, instead of species, due to functional redundancy expected from many soil microbial species (Barrios, 2007; van Bruggen and Semenov, 2000). Bacteria typically thrive in highly disturbed, low organic matter, nutrient-rich, environments.

Whereas fungi preferenvironments with less disturbance, high organic matter and low nutrient availability. Environment type is important in determining the fate of nutrients in a closed system (Heijden et al., 2008).

Ammonia-oxidizing archaea (AOA) and bacteria (AOB) are two critical microorganisms involved in autotrophic nitrification. AOA compared to AOB are oligotrophic microorganisms with tough cellular structures that can survive under nutrient or oxygen- depleted conditions. These organisms have been detected at 30ºC, the maximum temperature of survival for most microorganisms (Hatzenpichler, 2012). AOB are less prevalent than AOA, but some species have been found 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 universal 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 the measurement of microbial enzyme activity to understand their functions in soil environments (Nannipieri et al., 2002; Nielson and Windig, 2002). Microbial extracellular enzymes involved in nutrient cycling remain present in soil systems long after the microorganisms have decayed (Burns et al., 2013). Measuring soil enzymatic activity can expound on the potential activity of nutrient turnover, decomposition rates and other microbial activities of interest in soil (Nannipieri et al., 2002; Burns et al., 2013; van der Heijden et al. 2008). Extracellular enzymes such as phosphatase and urease are produced as a means to

obtain organically bound P and N (Sinsabaugh et al., 2002; van der Heijden et al. 2008).). These enzymes provide for the soil microbial community by signaling changes in nutrient availability and degrading organic material when the system is stressed (Burns et al., 2013; Arias et al. 2005; van der Heijden et al. 2008).

Soil respiration acts as another soil health indicator to estimate decomposition rates of soil organic matter (SOM) through carbon dioxide (CO_2) evolution (Kandeler, 2007). SOM consists of humic substances, plant, animal, and microbial biomass representative of all stages of life and is the largest terrestrial source of CO_2 (Kandeler, 2007; Schlesinger and Andrews, 2000). The stability of SOM is dependent on several biological and environmental factors, specifically by increased microbial populations and/or their activity (Schmidt et al., 2011; Kuzyakov et al., 2000; van der Heijden et al. 2008). Observing SOM turnover provides insight into the flow of energy and nutrients into a soil food web system which supplies mineralized nutrients to plants, stabilizes soil structure, and improves water retention, drainage, and cation exchange capacity (Barrios, 2007; van der Heijden et al. 2008).

Past studies have used microbial community abundance/composition, enzyme assays, and soil respiration as indicators in determining the relationship between soil quality and turfgrass systems. Mueller and Kussow (2005) observed that biostimulant products that included materials such as bacterial and fungal inoculants, yucca, seaweed extract, and several others did not affect soil 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). 17

Soil health indicators like microbial community composition, enzyme activity, and soil respiration have been used in recent turfgrass studies. However, the limited amount of research available between soil microbiology and golf course management elicits many questions about the connection among numerous aspects of both fields.

REFERENCES

Anderson, T. H., "Microbial Eco-Physiological Indicators to Assess Soil Quality." *Agriculture, Ecosystems and Environment*, vol. 98, no. 1–3, 2003, pp. 285–93, doi:10.1016/S0167-8809(03)00088-4.

Barrios, E. "Soil Biota, Ecosystem Services and Land Productivity." *Ecological Economics*, vol. 64, no. 2, 2007, pp. 269–85, doi:10.1016/j.ecolecon.2007.03.004.

Bartlett, M. D., et al. "Interactions between Microbial Community Structure and the Soil Environment Found on Golf Courses." *Soil Biology and Biochemistry*, vol. 39, no. 7, 2007, pp. 1533–41

Barton, L., and T. D. Colmer. "Ameliorating Water Repellency under Turfgrass of Contrasting Soil Organic Matter Content: Effect of Wetting Agent Formulation and Application Frequency." *Agricultural Water Management*, vol. 99, no. 1, Elsevier B.V., 2011, pp. 1–7, doi:10.1016/j.agwat.2011.08.009.

Beard, J. B., and R. L. Green. "The Role of Turfgrasses in Environmental Protection and Their Benefits to Humans." *Journal of Environmental Quality*, vol. 23, no. 3, 1994, pp. 452–60, doi:10.2134/jeq1994.00472425002300030007x.

Bonanomi, G., et al. "Organic Amendments, Beneficial Microbes, and Soil Microbiota: Toward a Unified Framework for Disease Suppression." *Annual Review of Phytopathology*, vol. 56, no. 1, 2018, pp. 1–20, doi:10.1146/annurev-phyto-080615-100046.

Burns, R. G., et al. "Soil Enzymes in a Changing Environment: Current Knowledge and Future Directions." *Soil Biology and Biochemistry*, vol. 58, Elsevier Ltd, 2013, pp.

216–34, doi:10.1016/j.soilbio.2012.11.009.

Chen, H., et al. “The Soil Microbial Community of Turf: Linear and Nonlinear Changes of Taxa and N-Cycling Gene Abundances over a Century-Long Turf Development.” *FEMS Microbiology Ecology*, vol. 95, no. 2, Oxford University Press, 2019, pp. 1–14, doi:10.1093/femsec/fiy224.

Chen, H., et al. “The Soil Microbial Community of Turf: Linear and Nonlinear Changes of Taxa and N-Cycling Gene Abundances over a Century-Long Turf Development.” *FEMS Microbiology Ecology*, vol. 95, no. 2, Oxford University Press, 2019, pp. 1–14, doi:10.1093/femsec/fiy224.

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

Cotton, S. “Wetting Agents: What Are They and How Do They Work?” *American Scientist*, vol. 106, no. 3, 2018, p. 138, doi:10.1511/2018.106.3.138.

Crecchio, C., et al. *Beneficial Soil Microbiome for Sustainable Agriculture Production*. no. September 2018, pp. 443–81, doi:10.1007/978-3-319-94232-2_9.

Cutler, N. R., et al. “Soil Health and Sustainability.” *Life Sciences*, vol. 62, no. 16, 1998, pp. 1433–41, doi:10.1016/S0024-3205(98)00082-4.

Das, S. K., and A. V., “Soil Enzymology.” *Soil Enzymology*, vol. 22, 2010, pp. 275–85, doi:10.1007/978-3-642-14225-3.

Dell, E. A., et al. “Heat Stress and N Fertilization Affect Soil Microbial and Enzyme Activities in the Creeping Bentgrass (Agrostis Stolonifera L.) Rhizosphere.” *Applied Soil Ecology*, vol. 56, 2012, pp. 19–26, doi:10.1016/j.apsoil.2012.02.002.

Diera, AA, Raymer, PL, Martinez-Espinoza, AD, Bauske, E, Habteselassie,

MY. Evaluating the impact of turf-care products on soil biological health. *J. Environ. Qual*. 2020; 49: 858– 868. https://doi.org/10.1002/jeq2.20080

du Jardin, P. "Plant Biostimulants: Definition, Concept, Main Categories and Regulation." *Scientia Horticulturae*, vol. 196, Elsevier B.V., 2015, pp. 3–14, doi:10.1016/j.scienta.2015.09.021.

Fierer, N., et al. "Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays." *Applied and Environmental Microbiology*, vol. 71, no. 7, 2005, pp. 4117–20, doi:10.1128/AEM.71.7.4117-4120.2005.

Fry, J., et al. *Reducing Fungicide Use on Creeping Bentgrass Greens*. no. July 2006, pp. 77–81.

González-vila, F. J., and A. S. Ball. *Soil Health — a New Challenge for Microbiologists And*. 2013, pp. 13–21.

Harman, G. E., et al. "Non-Target Effects of Fungicide Applications on Microbial Populations of Putting Greens." *USGA Green Sec. Rec.*, vol. 44, no. 4, 2006, pp. 9–12.

Hill, G. T., et al. "Methods for Assessing the Composition and Diversity of Soil Microbial Communities." *Applied Soil Ecology*, vol. 15, no. 1, 2000, pp. 25–36, doi:10.1016/S0929- 1393(00)00069-X.

Kennedy, A. C., and R. I. Papendick. *Microbial Characteristics of Soil Quality*. no. June, 1995, pp. 243–48.

Kokkoris, V., et al. "Site Specificity in Establishment of a Commercial Arbuscular Mycorrhizal Fungal Inoculant." *Science of the Total Environment*, vol. 660, Elsevier B.V., 2019, pp. 1135–43, doi:10.1016/j.scitotenv.2019.01.100.

Lee, J., et al. "Dollar Spot and Brown Patch Incidence in Creeping Bentgrass as Affected by

Acibenzolar-S-Methyl and Biostimulants." *HortScience*, vol. 38, no. 6, 2003, pp. 1223–26, doi:10.21273/hortsci.38.6.1223.

Liu, Y., et al. "Microbial and Soil Properties in Bentgrass Putting Greens: Impacts of Nitrogen Fertilization Rates." *Geoderma*, vol. 162, no. 1–2, Elsevier B.V., 2011, pp. 215–21, doi:10.1016/j.geoderma.2011.02.009.

Lo, C. T., et al. "Improved Biocontrol Efficacy of Trichoderma Harzianum 1295-22 for Foliar Phases of Turf Diseases by Use of Spray Applications." *Plant Disease*, vol. 81, no. 10, 1997, pp. 1132–38, doi:10.1094/PDIS.1997.81.10.1132.

Milesi, C., et al. "Mapping and Modeling the Biogeochemical Cycling of Turf Grasses in the United States." *Environmental Management*, vol. 36, no. 3, 2005, pp. 426–38, doi:10.1007/s00267-004-0316-2.

Moebius-Clune, B.N, et al. *Comprehensive Assessment of Soil Health*. 2016, http://www.nysaes.cornell.edu.

Mueller, S. R., and W. R. Kussow. "Biostimulant Influences on Turfgrass Microbial Communities and Creeping Bentgrass Putting Green Quality." *HortScience*, vol. 40, no. 6, 2005, pp. 1904–10, doi:10.21273/hortsci.40.6.1904.

Mukerji, K. G. "Soil Microbes." *Techniques in Mycorrhizal Studies*, vol. 50, 2002, pp. 7–13, doi:10.1007/978-94-017-3209-3_2.

Mundepi, A., et al. "Ammonia Oxidizers as Biological Health Indicators of Elevated Zn and Cu in Poultry Litter Amended Soil." *Water, Air, and Soil Pollution*, vol. 230, no. 10, Water, Air, & Soil Pollution, 2019, doi:10.1007/s11270-019-4283-x.

Nannipieri, P., et al. "Soil Enzyme Activity: A Brief History and Biochemistry as a Basis for Appropriate Interpretations and Meta-Analysis." *Biology and Fertility of Soils*, vol. 54,

no. 1, Biology and Fertility of Soils, 2018, pp. 11–19, doi:10.1007/s00374-017-1245-6.

Nielsen, M. N., and A. Winding. "Microorganisms as Indicators of Soil Health." *Neri*, vol. NERI Techn, no. 388, 2002, http://www.dmu.dk.

Sahrawat, K. L. (1984). Effects of temperature and moisture on urease activity in semi-arid tropical soils. Plant and Soil, 78(3), 401-408.

Saporta, R., et al. "A Method for a Fast Evaluation of the Biostimulant Potential of Different Natural Extracts for Promoting Growth or Tolerance against Abiotic Stress." *Agronomy*, vol. 9, no. 3, 2019, doi:10.3390/agronomy9030143.

Schlatter, D., et al. "Disease Suppressive Soils: New Insights from the Soil Microbiome." *Phytopathology*, vol. 107, no. 11, 2017, pp. 1284–97, doi:10.1094/PHYTO-03-17-0111- RVW.

Schloter, M., et al. "Indicators for Evaluating Soil Quality." *Agriculture, Ecosystems and Environment*, vol. 98, no. 1–3, 2003, pp. 255–62, doi:10.1016/S0167-8809(03)00085-9.

Sher, Y., et al. "Ammonia Transformations and Abundance of Ammonia Oxidizers in a Clay Soil Underlying a Manure Pond." *FEMS Microbiology Ecology*, vol. 81, no. 1, 2012, pp. 145–55, doi:10.1111/j.1574-6941.2012.01347.x.

Shi, W., et al. "Soil Microbial Community Composition and Function in Turfgrass Ecosystems." *Bioremediation, Biodiversity and Bioavailability*, vol. 1, no. 1, 2007, pp. 72–77.

Shi, W., et al. "Soil Enzyme Activities and Organic Matter Composition in a Turfgrass Chronosequence." *Plant and Soil*, vol. 288, no. 1–2, 2006, pp. 285–96, doi:10.1007/s11104-006-9116-1.

Shi, W., et al. “Soil Microbial Biomass, Activity and Nitrogen Transformations in a Turfgrass Chronosequence.” *Soil Biology and Biochemistry*, vol. 38, no. 2, 2006, pp. 311–19, doi:10.1016/j.soilbio.2005.05.008.

Shimada, M. “Agarose Gel Electrophoresis.” *Tanpakushitsu Kakusan Koso. Protein, Nucleic Acid, Enzyme*, vol. 41, no. 5, 1996, pp. 477–79.

Song, Enzhan, et al. “Influence of Repeated Application of Wetting Agents on Soil Water Repellency and Microbial Community.” *Sustainability (Switzerland)*, vol. 11, no. 16, 2019, pp. 1–13, doi:10.3390/su11164505.

Song, E., et al. “Influence of Repeated Application of Wetting Agents on Soil Water Repellency and Microbial Community.” *Sustainability (Switzerland)*, vol. 11, no. 16, 2019, pp. 1–13, doi:10.3390/su11164505.

Steketee, C. J., et al. “Effects of Genotype and Isolate on Expression of Dollar Spot in Seashore Paspalum.” *HortScience*, vol. 51, no. 1, 2016, pp. 67–73, doi:10.21273/hortsci.51.1.67.

Strandberg, M., et al. “Priorities for Sustainable Turfgrass Management: A Research and Industry Perspective.” *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, vol. 62, no. SUPPL.1, 2012, pp. 3–9, doi:10.1080/09064710.2012.682163.

Taliaferro, C. M. “Diversity and Vulnerability of Bermuda Turfgrass Species.” *Crop Science*, vol. 35, no. 2, 1995, p. 327, doi:10.2135/cropsci1995.0011183x003500020006x.

Thompson, G. L., and J. Kao-Kniffin. “Diversity Enhances NPP, N Retention, and Soil Microbial Diversity in Experimental Urban Grassland Assemblages.” *PLoS ONE*, vol. 11, no. 5, 2016, pp. 1–17, doi:10.1371/journal.pone.0155986.

Thompson, G. L., and J. Kao-Kniffin. “Urban Grassland Management Implications for Soil c and n Dynamics: A Microbial Perspective.” *Frontiers in Ecology and Evolution*, vol. 7, no. AUG, 2019, pp. 1–15, doi:10.3389/fevo.2019.00315.

Trabelsi, D, and R Mhamdi. “Microbial Inoculants and Their Impact on Soil Microbial Communities: A Review.” *BioMed Research International*, vol. 2013, 2013, doi:10.1155/2013/863240.

Trognitz, F., et al. “The Role of Plant-Microbiome Interactions in Weed Establishment and Control.” *FEMS Microbiology Ecology*, vol. 92, no. 10, 2016, pp. 1–15, doi:10.1093/femsec/fiw138.

Utobo, E. B. “Soil Enzymes as Bioindicators of Soil Ecosystem Status.” *Applied Ecology and Environmental Research*, vol. 13, no. 1, 2014, pp. 85–97, doi:10.15666/aeer/1301.

Van Bruggen, A. H. C., and A. M. Semenov. “In Search of Biological Indicators for Soil Health and Disease Suppression.” *Applied Soil Ecology*, vol. 15, no. 1, 2000, pp. 13–24, doi:10.1016/S0929-1393(00)00068-8.

Van Der Heijden, M G. A., et al. “The Unseen Majority: Soil Microbes as Drivers of Plant Diversity and Productivity in Terrestrial Ecosystems.” *Ecology Letters*, vol. 11, no. 3, 2008, pp. 296–310, doi:10.1111/j.1461-0248.2007.01139.x.

Wyngaard, N, et al. “Legacy Effect of Fertilization and Tillage Systems on Nitrogen Mineralization and Microbial Communities.” *Soil Science Society of America Journal*, vol. 80, no. 5, 2016, pp. 1262–71, doi:10.2136/sssaj2016.03.0070.

Yakhin, O. I., et al. “Biostimulants in Plant Science: A Global Perspective.” *Frontiers in Plant Science*, vol. 7, 2017, doi:10.3389/fpls.2016.02049.

Yao, H, et al. “Soil Microbial Community Structure and Diversity in a Turfgrass Chronosequence: Land-Use Change versus Turfgrass Management.” *Applied Soil Ecology*, vol. 34, no. 2–3, 2006, pp. 209–18, doi:10.1016/j.apsoil.2006.01.009.

Zontek, B. Y. Stanley J. *Understanding the Different Wetting Agent Chemistries*. Green Record, vol. 50 2012, pp. 1–6.

CHAPTER THREE

EVALUATING THE IMPACT OF WETTING AGENTS AND PLANT GROWTH REGULATORS ON TURF QUALITY AND SOIL BIOLOGICAL HEALTH[1]

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ABSTRACT

Golf course superintendents face pressure to maintain a thriving green while also promoting more environmentally friendly practices. Among the most commonly products to maintain golf course greens are wetting agents (WAs) and plant growth regulators (PGRs). However, the impact of these products on soil biological health is not clear. A yearlong field study was conducted to determine the impacts of select WAs and PGRs on turf quality and soil biological health. Turf quality was measured by taking images of the experimental plots with a digital camera mounted on a lightbox, followed by the images being analyzed to determine the percent green cover. As indicators of soil biological health, the abundance of ammonia-oxidizers, soil respiration and enzyme activities were determined. The WAs and PGRs did not significantly impact the abundance of ammonia-oxidizers or soil respiration. Revolution from WAs and Cutless and Trimmit from PGRs improved phosphatase activity. WAs C+D and 16-19 temporarily suppressed urease activity while Revolution stimulated it after multiple applications. The impact of Cutless on urease activity was positive but shorted lived. The positive impacts of these treatments were probably because of their impact in improving soil moisture availability (WAs) and root growth (PGRs). None of the WAs improved turf quality while the PGRs Primo Maxx and Trimmit improved turf quality after multiple applications. A strong correlation was noted between soil respiration and turf quality. Overall, the WAs and PGRs had positive impact on soil biological health.

INTRODUCTION

Due to the pressure to maintain high quality turf under climatic, pest and use-induced stresses, superintendents use several turfcare products. Among the most commonly used products are wetting agents and plant growth regulators (Karnok et al., 2004). However, the impacts of these products on soil microbial communities are not clear.

Wetting agents are used to address the problem of localized dry spots (LDS) prevalent in turfgrass soils during the summer and caused by soil water repellency. The occurrence of LDS causes water stress and negatively affects turf quality. Despite differences among products, several studies have reported wetting agents to be effective in reducing LDS in golf courses.

However, some wettings agents can cause phytotoxicity in turf and require irrigation immediately following application to minimize turf damage (Karnok, 2006). The effect of wetting agents on the turfgrass soil microbial communities is unknown. Some studies have reported the inhibition of microbially mediated decomposition of pollutants due to surfactants in non-turfgrass soils, with subsequent changes in microbial populations (e.g., Laha and Luthy, 1991).

Plant growth regulators are used to promote healthier turf with the ability to withstand various types of stresses. Growth regulators are designed to slow down production of hormones (e.g., gibberellic acid) thereby minimizing vertical shoot growth while promoting lateral and below-ground root growth. There are several studies that tested their efficacy on turfgrass growth and quality with mixed results (McCann and Huang, 2007; Gardner and Wherley, 2005) but their impact on the turfgrass soil microbial communities has not been

examined. It is important to study whether these products have similar inhibition effect on the microorganisms, and what the implications would for their use in turfgrass system.

Biological soil health describes the capacity of a soil to provide essential benefits to turfgrasses. These include disease suppression, decomposition of organic matter, increased nutrient availability, and more. These benefits are all facilitated by microbial communities within a soil system. Parameters such as microbial abundance, enzyme activity, and soil respiration can serve as indicators of biological soil health. Microbial abundance and diversity are viable soil quality measurements because microbial communities are heavily influenced by different types of land use and ecosystem flora (Doran and Zeiss, 2000; Yao et al., 2000; Nielson & Winding, 2002). Ammonia-oxidizing archaea (AOA) and bacteria (AOB) are microbial groups involved in autotrophic nitrification, the first step in ammonia (NH_3) oxidation, in a soil system and are highly sensitive to changes caused by management practices (Hatzenpichler, 2012; Norton and Stark, 2011; Norton, 2011). Measuring enzyme activity provides deeper explanation of microbial processes including those related to nutrient cycling (Barrios, 2007; Kandeler, 2007; Shi et al., 2006). Phosphatase and urease are two such enzymes that are microbially-secreted as a means to mineralize organically bound phosphorus P and N (Plante, 2007; Mobley and Hausinger, 1989; Kandeler, 2007). Rates of soil respiration is often used as a measure of microbial activity as it is indicative of soil organic matter decomposition that produces CO_2 evolution. It helps provide insight into energy flow within a soil food web (Kandeler, 2007; Barrios, 2007; M. Nielson & A. Winding, 2002).

The focus of this study is to determine the impacts of wetting agents and plant growth regulators on biological soil health and turf quality and discern the relationship

between soil health and turf quality.

METHODS AND MATERIALS

Study Sites

Two field trials were established in Spring 2018, one on a fairway at Rivermont Golf Club in Johns Creek, GA and one on a putting green at the University of Georgia Campus in Griffin, GA. Total rainfall over the fairway (Tifway Bermudagrass) and putting green (A1-A4 Creeping Bentgrass) trials was 68 cm and 70 cm respectively; average temperature ranged from 16°C to 28°C in the fairway trial and 16°C to 28°C in the putting green trial (AEMN, 2019). A sensor and CR1000 datalogger (Campbell Scientific, Logan UT) was installed at the Rivermont fairway location to capture soil temperature and moisture. Average soil temperature ranged from 22°C to 30°C in Rivermont and 24°C to 30°C in Griffin (AEMN 2019). Average volumetric soil moisture ranged from 7% to 12% in the putting green trial and 24%-35% in the fairway trial (AEMN 2019).

Wetting Agent Trial

Field plots were established on research greens (A1-A4 Creeping Bentgrass) at the University of Georgia Griffin Campus in May 2018. Plots (3 x 1.5 m) were arranged in a randomized complete block design with five replications (Figure 3.1). Four treatments (three wetting agents and a non- treated control) were applied to experimental plots on a research green sandy soil with 3% soil organic matter (SOM) and an average pH in water of 6.2. Wetting agent treatment included Cascade Plus® and Duplex® (C+D), Revolution® (Rev), and Sixteen 90® (16-90) (Table 3.1). Product samples were sent to UGA's Agricultural and Environmental Services Laboratory (http://aesl.ces.uga.edu) for analysis on organic matter

and nutrient contents (nitrogen, phosphorous, carbon) (Table 3.1). To assure uniformity of application, treatments were applied twice at half recommended treatment rates with a 15-L backpack sprayer. Experimental plot dimensions were used to calculate total surface area needed to provide double coverage for five replicates of one treatment after calibration of backpack sprayer. Applications were repeated once a month after initial treatment for a total of 10 total applications.

Turfgrass was maintained based on a general upkeep schedule. This included the application of N fertilization in combination with fungicide for pathogen protection. Nitrogen was applied at 0.367 kg/100 m^2 every three weeks. Daconil, Banol, and Affirm were used for mitigating fungal diseases throughout the duration of the project. Turf was maintained at 3.12- 2.67 mm mowing height and received irrigation three times daily.

Plant Growth Regulator Trial

Field plots were established on a fairway (Tifway Bermudagrass) at Rivermont Golf Club in John's Creek, GA in May 2018. Four treatments (Primo Maxx®, Cutless®, Trimmit®, and a non-treated control) were applied to 3 m x 1.5 m plots on sandy clay loam fairway soil with an average pH of 5.7 (Figure 3.2). As with wetting agents, PGRs were sent to UGA's Agricultural and Environmental Services Lab for nutrient analysis (Table 3.1). Each treatment was replicated five times and applied as described in the wetting agent trial at the recommended rate using half field rates to ensure adequate coverage with a backpack sprayer. Treatments were applied once monthly after initial application for a total of 12 applications.

As part of turf management, the plots received 0.567 kg urea in combination with 0.227 kg soft rock phosphate. Mowing height was maintained at 12.7 mm and fairway

was irrigated in accordance with superintendent recommendations.

Sampling

Composite samples of 6 to 7 soil cores were collected from the research greens using a 127-mm soil probe to collect 10-cm soil columns from the greens. Similarly, composite samples were collected from the fairway using a drill auger and bucket to obtain 10-cm soil columns. Soil samples were kept at 4°C until processed through a 2-mm sieve to remove plant material. Sieved samples were then used for measuring microbial abundance, soil respiration, and enzyme assays. Five grams of each soil sample were placed in separate Ziploc bags and stored at -20°C for DNA extraction and quantitative polymerase chain reaction analysis. Dry soil weights were determined gravimetrically by placing soil samples in an oven at 100°C for 24 h and cooling in a desiccator for 2 h. Moisture content of all samples was calculated and further used to calculate the oven dry soil weight (g) equivalent of the amount used for analysis.

Sample Analysis

Quantitative polymerase chain reaction (qPCR) was used to determine the abundance of total bacteria, total fungi, ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) in soil samples. Soil DNA was extracted from all samples using the DNeasy PowerSoil DNA extraction kit (Qiagen, Germantown, MD). Table 3.2 summarizes target genes, amplicon lengths, primers, and thermal cycling conditions. Reaction volume for qPCR was 20 µL containing 10 µL PowerUp SYBR Green Master Mix (ThermoFisher Scientific, Grand Island, New York), 2 µL DNA template, 0.8 µL of both forward and reverse primers, and 6.4 µL nuclease-free PCR water; all reactions

were run in duplicate. Standards for target organisms ranging from 30 to 3 x 10^5 copies of DNA per µL were prepared as standards and run-in triplicate for all assays (ThermoFisher Scientific, Grand Island, New York). The standards were prepared as described in Mundepi et al (2017). StepOne Plus Software (Applied Biosystems, city, state) was used to analyze qPCR data.

Soil sample extracts were colorimetrically analyzed to estimate the rate of phosphatase activity as an indicator of soil P cycling as described in Tabatahai (1994). For urease assay, soil samples were analyzed using a 2% boric acid trap method to estimate the rate of urease activity as an indicator of soil N cycling as described in Mobley and Hausinger (1989). Three of the five replicates from each treatment were selected to examine the effects of treatments on soil respiration as an indicator of microbial activity as described in Zibilske (1994).

Turf quality was assessed by taking images of the plots with a digital camera and analyzing the images with the Assess 2.0 image analysis software (American Phytopathological Society) as percent green cover (ratio of green to total pixels). This provides an objective assessment of the overall turf quality and quantitative data for robust statistical analysis.

Statistical Analysis

The data were summarized into descriptive statistics (e.g., mean and standard errors). Analysis of variance was carried out to test the statistical significance of the effects of the wetting agents and plant growth regulators on turf quality and indicators of soil health at $\alpha = 0.05$ in JMP 14. Treatment was a categorical variable while sampling day was a continuous

variable, and experiment plot was treated as random effect. Correlation analysis using pairwise comparisons was done to determine the relationship between soil health parameters and turf quality. Tukey's honest significant difference (HSD) test was used to conduct post hoc analyses to identify significant relationships among treatments within all models.

RESULTS AND DISCUSSION

Microbial Abundance

The abundances of AOB and AOA were not significantly affected by wettings agents or plant growth regulators in all of the sampling times. There were greater numbers of AOB than AOA present in both the WA and PGR trials. AOB abundance ranged from 3.74 log copies g^{-1} to 4.82 log copies g^{-1} and AOA counts ranged from 3.01 log copies g^{-1} to 4.17 log copies g^{-1} in the WA trial (Table 3.3). In the PGR trial, AOB ranged from 5.79 log copies g^{-1} to 6.42 log copies g^{-1}, and AOA ranged from 3.49 log copies g^{-1} to 4.12 log copies g^{-1} (Table 3.4). Between each sampling date there appeared to be a decrease in overall abundance over time. This same phenomenon was also seen in the PGR trial and might be related to changes in weather and management practices over time. 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 nutrient availability (Beirn et al., 2017; Elliott et al., 2008; Bartlett et al., 2009; Bigelow et al., 2002; Kuramae et al., 2010).

AOB generally dominate under conditions in which nitrogen is readily available in systems that are highly managed, and become more competitive than AOA (Hatzenpichler, 2012). This might explain why they were more abundant than AOA in both trials. 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).

It is important to note that these results are reflective of short-term impacts, a longer study including more treatment and sampling times could yield entirely different results. The use of these products showed no negative impacts on the abundance of ammonia oxidizing microorganisms, meaning they do not appear to hinder or enhance their presence.

Soil Respiration

There were no significant treatment effects observed for each sampling time. In the WA trial, there was an increase in soil respiration between the first and second sampling times, with greater rates observed after three applications (Table 3.5). However, rates of the third sampling date returned to match those of the initial one year later. The spike in soil respiration seen in the July 2018 indicated microbial activity responses to the environment. Soil respiration becomes more responsive when soil temperature and moisture content increase (Phillips and Nickerson, 2015). An increase in temperature would drive up respiration rate which is the likely reason for the spike seen in July. Throughout the duration of the study microbial activity stabilized to levels similar to those at the beginning of the trial.

Similar to WA trial, soil respiration was not significantly affected by plant growth regulators on both sampling times that represented one- and seven-time applications (Table 3.5). The only difference was between the two sampling times in which soil respiration rates decreased between July 27, 2018 and June 17, 2019 after 7 applications. Soil respiration ranged from 1.81 mg CO_2 g^{-1} to 2.06 mg CO_2 g^{-1}1 in the WA trial and 1.80 CO_2 $g^{-1}$to 2.4 mg

CO_2 g^{-1}in the PGR trial (Table 3.5 and 3.6, respectively). These respiration rates are greater than those observed in a similar study conducted on the fairway one year prior (Dierra et. al, 2020). This could be due to weather and management related changes between the two study times. The lack of significant impacts in either trial can likely be attributed to the soil already having sufficient carbon. Adding more carbon sources in small amounts over a short period of time will less likely impact the soil enough to make a difference.

Phosphatase Activity

In the WA trial, there was a significant treatment effect on phosphatase activities in the first sampling date, which is 24 h after the first application. Revolution was the only WA that resulted in significantly greater activity than the Control (Table 3.7). However, there were no treatment effects on phosphatase activity on the second and third sampling dates, three and six applications later, respectively (Table 3.7). The addition of wetting agent, Revolution, might have allowed water and nutrients from fertilization to become more permeable throughout the soil and therefore more available to the microorganisms (Zontek, 2012). Revolution is a modified methyl block co-polymer whereas the other products are block co-polymers (Zontek, 2012). This modification in the product changes how the surfactant interacts with the hydrophobic soil covering, giving an overall better air-to-water ration in the soil (Zontek, 2012). For WAs that did not enhance phosphatase activity, a plausible reason is that they could not increase microbial activity that would lead to increased production of phosphatase. The impacts seen in the first sampling time as compared to the second and third indicate the temporary nature of the impact.

In the PGR trial, there were significant differences between Cutless and Control, with

Cutless resulting in significantly higher phosphatase activity than the Control after first application of the products (Table 3.8). After multiple applications, both Trimmit and the Cutless resulted in significantly higher phosphatase activity than the control and Primo Maxx. It is possible that both Cutless and TRIM might have impacted phosphatase activity indirectly through their effect on root growth, which would promote the production of labile organic substrates that will in turn promote microbial activity. However, unlike Trimmit, the impact of Cutless was both immediate (after one-time application) and long-lasting (after multiple applications).

Overall, phosphatase activity ranged from 1.26 µmol P evolved g^{-1} h^{-1} to 1.99 µmol P evolved g^{-1} h^{-1} in the WA trial (Table 3.7). In the PGR trial, phosphatase activity ranged from 1.62 µmol P evolved g^{-1} h^{-1} to 8.81 µmol P evolved g^{-1} h^{-1} (Table 3.8). Higher overall rates of phosphatase activity in the PGR trial compared to the WA trial is likely associated with less disturbance, higher levels of organic material, and a higher percentage of in the fairway. These effects were also seen in a previous study conducted using the same products (Dierra et. al, 2020).

Urease Activity

In the WA trial, C+D and 16-19 resulted in significant smaller levels of urease activity than the Control 24 h after the first-time application (Table 3.7). After multiple applications of the products (July 2, 2018), REV was the only product that resulted in significantly greater urease activity than another WA (C+D) and the Control. These results suggest two things. First, C+D and 16-19 initially slowed down urease activity. Second, REV increased urease activity after multiple application, indicating its cumulative impact over

time. This suggests REV stimulated ureolytic microbial activity in the green. Urea is hydrolyzed by water and urease enzymes (Killham and Prosser, 2007). The removal of hydrophobic organic material by REV and increased soil permeability may have released urease enzymes attached to the soil colloids and increased water flow throughout the soil profile (Kostka, 2000; Burns et al., 2013). This WA most likely enhanced the infiltration of water into the soil, increasing the moisture content of the soil and in doing so increasing urease activity. (Sahrawat and Soil, 1984).

In the PGR trial, Cutless significantly increased urease activity as compared to the Control but was not significantly different from the other PGRs (Table 3.8) after one-time application. However, multiple applications of the products did not result in any significant differences between the products and the Control. This indicates the temporary nature of the impact of Cutless.

Overall, urease activity was much greater in the fairway than the greens, indicating the difference in microbial activity between the two systems. Urease activity ranged from 2.43 to 4.24 $NH_3\ g^{-1}\ h^{-1}$ in the research greens (Table 3.7), and from 34.54 to 42.42 µmol NH_3 evolved $g^{-1}\ h^{-1}$ in the fairway trial (Table 3.8). This mirrors what was observed with phosphatase activity too.

Turf Quality

Turf quality ranged from 100 to 87 percent green cover in the WA trial and from 76 to 93 percent green coverage in the PGR trial (Table 3.8 and 3.9, respectively). In the WA trial, there were no significant differences in turf quality among the treatments. Over time, the turf quality deteriorated in all of the plots (Table 3.8). Much of this was due to the greens being overrun with

weeds and disease. The problem in the REV and 16-90 receiving plots was exacerbated as these products resulted in burn that caused observed phytotoxicity.

In the fairway PGR trial, there were no initial significant differences between the treatments. However, after multiple applications PM and TRIM improved turf quality (Table 3.9). This is consistent with findings of Glab et. al (2020) that noted PGRs containing Paclobutrazol and Trinexpac Ethyl produced overall improvements in turfgrass quality and color.

Correlation analysis was done to study the relationship between soil respiration, soil enzyme activities and turf quality. This resulted in a strong positive linear relationship between turf quality and soil respiration ($r = 0.7578$; $p = <.0001$) in the WA trial and a moderate negative linear relationship between turf quality and soil respiration ($r = -0.5171$; $p = 0.0014$) in the PGR trial.

In future studies, long-term responses over several years would probably be able to provide more insight on the sustainability and longevity of the use of these products in golf course management programs as it relates to the microbiology of these soils.

SUMMARY AND CONCLUSIONS

Overall, the WAs and PGRs did not significantly impact the abundance of AOB and AOA or soil respiration. The impact of the treatments on soil biological health was mainly reflected on soil enzyme activities. Revolution from WAs and Cutless and Trimmit from PGRs resulted in a significant increase in phosphatase activity. Urease activity, on the other hand, was initially suppressed by WAs C+D and 16-19 while it was stimulated by Revolution after multiple applications. The impact of Cutless on urease activity was positive but short-lived. The positive impacts of these treatments were probably due to their effect in improving soil moisture

availability (WAs) and root growth (PGRs), which would in turn improve microbial activity as reflected by the enzyme activities. When negative impacts of some WAs were observed (i.e., on urease) they were temporary. Some WAs were observed to cause phytotoxicity on the turf and might have also negatively impacted microbial activity. None of the WAs improved turf quality as compared to the Control at both sampling times. The PGRs Primo Maxx and Trimmit, on the other hand, significantly improved turf quality after multiple applications.

This study presents some insight into the dynamics between turf care products and soil microbial communities in golf course soils and the relationships between these indicators and turf quality. The practical importance of the study is the information provided to golf course superintendents and turfgrass managers enabling them to make management decisions that improve the sustainability and survivability of their turf systems.

REFERENCES

Anderson, T. H., "Microbial Eco-Physiological Indicators to Assess Soil Quality." *Agriculture, Ecosystems and Environment*, vol. 98, no. 1–3, 2003, pp. 285–93, doi:10.1016/S0167-8809(03)00088-4.

Barrios, E. "Soil Biota, Ecosystem Services and Land Productivity." *Ecological Economics*, vol. 64, no. 2, 2007, pp. 269–85, doi:10.1016/j.ecolecon.2007.03.004.

Bartlett, M. D., et al. "Interactions between Microbial Community Structure and the Soil Environment Found on Golf Courses." *Soil Biology and Biochemistry*, vol. 39, no. 7, 2007, pp. 1533–41

Barton, L., and T. D. Colmer. "Ameliorating Water Repellency under Turfgrass of Contrasting Soil Organic Matter Content: Effect of Wetting Agent Formulation and Application Frequency." *Agricultural Water Management*, vol. 99, no. 1, Elsevier B.V., 2011, pp. 1–7, doi:10.1016/j.agwat.2011.08.009.

Beard, J. B., and R. L. Green. "The Role of Turfgrasses in Environmental Protection and Their Benefits to Humans." *Journal of Environmental Quality*, vol. 23, no. 3, 1994, pp. 452–60, doi:10.2134/jeq1994.00472425002300030007x.

Bonanomi, G., et al. "Organic Amendments, Beneficial Microbes, and Soil Microbiota: Toward a Unified Framework for Disease Suppression." *Annual Review of Phytopathology*, vol. 56, no. 1, 2018, pp. 1–20, doi:10.1146/annurev-phyto-080615-100046.

Burns, R. G., et al. "Soil Enzymes in a Changing Environment: Current Knowledge and Future Directions." *Soil Biology and Biochemistry*, vol. 58, Elsevier Ltd, 2013, pp.

216–34, doi:10.1016/j.soilbio.2012.11.009.

Chen, H., et al. “The Soil Microbial Community of Turf: Linear and Nonlinear Changes of Taxa and N-Cycling Gene Abundances over a Century-Long Turf Development.” *FEMS Microbiology Ecology*, vol. 95, no. 2, Oxford University Press, 2019, pp. 1–14, doi:10.1093/femsec/fiy224.

Chen, H., et al. “The Soil Microbial Community of Turf: Linear and Nonlinear Changes of Taxa and N-Cycling Gene Abundances over a Century-Long Turf Development.” *FEMS Microbiology Ecology*, vol. 95, no. 2, Oxford University Press, 2019, pp. 1–14, doi:10.1093/femsec/fiy224.

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

Cotton, S. “Wetting Agents: What Are They and How Do They Work?” *American Scientist*, vol. 106, no. 3, 2018, p. 138, doi:10.1511/2018.106.3.138.

Crecchio, C., et al. *Beneficial Soil Microbiome for Sustainable Agriculture Production*. no. September 2018, pp. 443–81, doi:10.1007/978-3-319-94232-2_9.

Cutler, N. R., et al. “Soil Health and Sustainability.” *Life Sciences*, vol. 62, no. 16, 1998, pp. 1433–41, doi:10.1016/S0024-3205(98)00082-4.

Das, S. K., and A. V., “Soil Enzymology.” *Soil Enzymology*, vol. 22, 2010, pp. 275–85, doi:10.1007/978-3-642-14225-3.

Dell, E. A., et al. “Heat Stress and N Fertilization Affect Soil Microbial and Enzyme Activities in the Creeping Bentgrass (Agrostis Stolonifera L.) Rhizosphere.” *Applied Soil Ecology*, vol. 56, 2012, pp. 19–26, doi:10.1016/j.apsoil.2012.02.002.

Diera, AA, Raymer, PL, Martinez-Espinoza, AD, Bauske, E, Habteselassie,

MY. Evaluating the impact of turf-care products on soil biological health. *J. Environ. Qual*. 2020; 49: 858– 868. https://doi.org/10.1002/jeq2.20080

du Jardin, P. "Plant Biostimulants: Definition, Concept, Main Categories and Regulation." *Scientia Horticulturae*, vol. 196, Elsevier B.V., 2015, pp. 3–14, doi:10.1016/j.scienta.2015.09.021.

Fierer, N., et al. "Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays." *Applied and Environmental Microbiology*, vol. 71, no. 7, 2005, pp. 4117–20, doi:10.1128/AEM.71.7.4117-4120.2005.

Fry, J., et al. *Reducing Fungicide Use on Creeping Bentgrass Greens*. no. July 2006, pp. 77–81.

González-vila, F. J., and A. S. Ball. *Soil Health — a New Challenge for Microbiologists And*. 2013, pp. 13–21.

Harman, G. E., et al. "Non-Target Effects of Fungicide Applications on Microbial Populations of Putting Greens." *USGA Green Sec. Rec.*, vol. 44, no. 4, 2006, pp. 9–12.

Hill, G. T., et al. "Methods for Assessing the Composition and Diversity of Soil Microbial Communities." *Applied Soil Ecology*, vol. 15, no. 1, 2000, pp. 25–36, doi:10.1016/S0929- 1393(00)00069-X.

Kennedy, A. C., and R. I. Papendick. *Microbial Characteristics of Soil Quality*. no. June, 1995, pp. 243–48.

Kokkoris, V., et al. "Site Specificity in Establishment of a Commercial Arbuscular Mycorrhizal Fungal Inoculant." *Science of the Total Environment*, vol. 660, Elsevier B.V., 2019, pp. 1135–43, doi:10.1016/j.scitotenv.2019.01.100.

Lee, J., et al. "Dollar Spot and Brown Patch Incidence in Creeping Bentgrass as Affected by

Acibenzolar-S-Methyl and Biostimulants." *HortScience*, vol. 38, no. 6, 2003, pp. 1223–26, doi:10.21273/hortsci.38.6.1223.

Liu, Y., et al. "Microbial and Soil Properties in Bentgrass Putting Greens: Impacts of Nitrogen Fertilization Rates." *Geoderma*, vol. 162, no. 1–2, Elsevier B.V., 2011, pp. 215–21, doi:10.1016/j.geoderma.2011.02.009.

Lo, C. T., et al. "Improved Biocontrol Efficacy of Trichoderma Harzianum 1295-22 for Foliar Phases of Turf Diseases by Use of Spray Applications." *Plant Disease*, vol. 81, no. 10, 1997, pp. 1132–38, doi:10.1094/PDIS.1997.81.10.1132.

Milesi, C., et al. "Mapping and Modeling the Biogeochemical Cycling of Turf Grasses in the United States." *Environmental Management*, vol. 36, no. 3, 2005, pp. 426–38, doi:10.1007/s00267-004-0316-2.

Moebius-Clune, B.N, et al. *Comprehensive Assessment of Soil Health*. 2016, http://www.nysaes.cornell.edu.

Mueller, S. R., and W. R. Kussow. "Biostimulant Influences on Turfgrass Microbial Communities and Creeping Bentgrass Putting Green Quality." *HortScience*, vol. 40, no. 6, 2005, pp. 1904–10, doi:10.21273/hortsci.40.6.1904.

Mukerji, K. G. "Soil Microbes." *Techniques in Mycorrhizal Studies*, vol. 50, 2002, pp. 7–13, doi:10.1007/978-94-017-3209-3_2.

Mundepi, A., et al. "Ammonia Oxidizers as Biological Health Indicators of Elevated Zn and Cu in Poultry Litter Amended Soil." *Water, Air, and Soil Pollution*, vol. 230, no. 10, Water, Air, & Soil Pollution, 2019, doi:10.1007/s11270-019-4283-x.

Nannipieri, P., et al. "Soil Enzyme Activity: A Brief History and Biochemistry as a Basis for Appropriate Interpretations and Meta-Analysis." *Biology and Fertility of Soils*, vol. 54,

no. 1, Biology and Fertility of Soils, 2018, pp. 11–19, doi:10.1007/s00374-017-1245-6.

Nielsen, M. N., and A. Winding. "Microorganisms as Indicators of Soil Health." *Neri*, vol. NERI Techn, no. 388, 2002, http://www.dmu.dk.

Sahrawat, K. L. (1984). Effects of temperature and moisture on urease activity in semi-arid tropical soils. Plant and Soil, 78(3), 401-408.

Saporta, R., et al. "A Method for a Fast Evaluation of the Biostimulant Potential of Different Natural Extracts for Promoting Growth or Tolerance against Abiotic Stress." *Agronomy*, vol. 9, no. 3, 2019, doi:10.3390/agronomy9030143.

Schlatter, D., et al. "Disease Suppressive Soils: New Insights from the Soil Microbiome." *Phytopathology*, vol. 107, no. 11, 2017, pp. 1284–97, doi:10.1094/PHYTO-03-17-0111- RVW.

Schloter, M., et al. "Indicators for Evaluating Soil Quality." *Agriculture, Ecosystems and Environment*, vol. 98, no. 1–3, 2003, pp. 255–62, doi:10.1016/S0167-8809(03)00085-9.

Sher, Y., et al. "Ammonia Transformations and Abundance of Ammonia Oxidizers in a Clay Soil Underlying a Manure Pond." *FEMS Microbiology Ecology*, vol. 81, no. 1, 2012, pp. 145–55, doi:10.1111/j.1574-6941.2012.01347.x.

Shi, W., et al. "Soil Microbial Community Composition and Function in Turfgrass Ecosystems." *Bioremediation, Biodiversity and Bioavailability*, vol. 1, no. 1, 2007, pp. 72–77.

Shi, W., et al. "Soil Enzyme Activities and Organic Matter Composition in a Turfgrass Chronosequence." *Plant and Soil*, vol. 288, no. 1–2, 2006, pp. 285–96, doi:10.1007/s11104-006-9116-1.

Shi, W., et al. “Soil Microbial Biomass, Activity and Nitrogen Transformations in a Turfgrass Chronosequence.” *Soil Biology and Biochemistry*, vol. 38, no. 2, 2006, pp. 311–19, doi:10.1016/j.soilbio.2005.05.008.

Shimada, M. “Agarose Gel Electrophoresis.” *Tanpakushitsu Kakusan Koso. Protein, Nucleic Acid, Enzyme*, vol. 41, no. 5, 1996, pp. 477–79.

Song, Enzhan, et al. “Influence of Repeated Application of Wetting Agents on Soil Water Repellency and Microbial Community.” *Sustainability (Switzerland)*, vol. 11, no. 16, 2019, pp. 1–13, doi:10.3390/su11164505.

Song, E., et al. “Influence of Repeated Application of Wetting Agents on Soil Water Repellency and Microbial Community.” *Sustainability (Switzerland)*, vol. 11, no. 16, 2019, pp. 1–13, doi:10.3390/su11164505.

Steketee, C. J., et al. “Effects of Genotype and Isolate on Expression of Dollar Spot in Seashore Paspalum.” *HortScience*, vol. 51, no. 1, 2016, pp. 67–73, doi:10.21273/hortsci.51.1.67.

Strandberg, M., et al. “Priorities for Sustainable Turfgrass Management: A Research and Industry Perspective.” *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, vol. 62, no. SUPPL.1, 2012, pp. 3–9, doi:10.1080/09064710.2012.682163.

Taliaferro, C. M. “Diversity and Vulnerability of Bermuda Turfgrass Species.” *Crop Science*, vol. 35, no. 2, 1995, p. 327, doi:10.2135/cropsci1995.0011183x003500020006x.

Thompson, G. L., and J. Kao-Kniffin. “Diversity Enhances NPP, N Retention, and Soil Microbial Diversity in Experimental Urban Grassland Assemblages.” *PLoS ONE*, vol. 11, no. 5, 2016, pp. 1–17, doi:10.1371/journal.pone.0155986.

Thompson, G. L., and J. Kao-Kniffin. “Urban Grassland Management Implications for Soil c and n Dynamics: A Microbial Perspective.” *Frontiers in Ecology and Evolution*, vol. 7, no. AUG, 2019, pp. 1–15, doi:10.3389/fevo.2019.00315.

Trabelsi, D, and R Mhamdi. “Microbial Inoculants and Their Impact on Soil Microbial Communities: A Review.” *BioMed Research International*, vol. 2013, 2013, doi:10.1155/2013/863240.

Trognitz, F., et al. “The Role of Plant-Microbiome Interactions in Weed Establishment and Control.” *FEMS Microbiology Ecology*, vol. 92, no. 10, 2016, pp. 1–15, doi:10.1093/femsec/fiw138.

Utobo, E. B. “Soil Enzymes as Bioindicators of Soil Ecosystem Status.” *Applied Ecology and Environmental Research*, vol. 13, no. 1, 2014, pp. 85–97, doi:10.15666/aeer/1301.

Van Bruggen, A. H. C., and A. M. Semenov. “In Search of Biological Indicators for Soil Health and Disease Suppression.” *Applied Soil Ecology*, vol. 15, no. 1, 2000, pp. 13–24, doi:10.1016/S0929-1393(00)00068-8.

Van Der Heijden, M G. A., et al. “The Unseen Majority: Soil Microbes as Drivers of Plant Diversity and Productivity in Terrestrial Ecosystems.” *Ecology Letters*, vol. 11, no. 3, 2008, pp. 296–310, doi:10.1111/j.1461-0248.2007.01139.x.

Wyngaard, N, et al. “Legacy Effect of Fertilization and Tillage Systems on Nitrogen Mineralization and Microbial Communities.” *Soil Science Society of America Journal*, vol. 80, no. 5, 2016, pp. 1262–71, doi:10.2136/sssaj2016.03.0070.

Yakhin, O. I., et al. “Biostimulants in Plant Science: A Global Perspective.” *Frontiers in Plant Science*, vol. 7, 2017, doi:10.3389/fpls.2016.02049.

Yao, H, et al. “Soil Microbial Community Structure and Diversity in a Turfgrass Chronosequence: Land-Use Change versus Turfgrass Management.” *Applied Soil Ecology*, vol. 34, no. 2–3, 2006, pp. 209–18, doi:10.1016/j.apsoil.2006.01.009.

Zontek, B. Y. Stanley J. *Understanding the Different Wetting Agent Chemistries*. Green Record, vol. 50 2012, pp. 1–6.

Table 3.1. Description of the wettings agents (WA) and plant growth regulators (PGR) used in the study.

Treatment	Product Type	Trade Name	Manufacturer	Active Ingredient (a.i.)	Rate of Application	Trial Location	% C & N
					—L a.i. ha^{-1}—		
C+D		Cascade Plus	Precision Laboratories	Alcohol ethoxylates	1.3		63, 0.02
				Polyethylene polypropylene glycol block copolymer	11		
	Wetting Agent	Duplex	Precision Laboratories	Alcohol ethoxylates	0.11	Griffin Research Greens	14, 0.45
				Alkyl aryl sulfonate	0.02		
				Ethylenediaminetetraacetic acid	0.05		
REV		Revolution	Aquatrols	Modified alkylated polyol	9.6		65, 0.05
16-90		Sixteen 90	Aquatrols	Propoxylated Polyethylene Glycols	9.6		63, 0.02
PM		Primo Maxx	Syngenta	Trinexapac-ethyl	0.13		N/A
CL	Plant Growth Regulator	Cutless	SePRO	Flurprimidol	0.29	Rivermont Fairway	67, 1.43
TRIM		Trimmit	Syngenta	Paclobutrazol	0.13		20,
3.6							

Table 3.2. Target genes, amplicon lengths, primer sequences, and thermal cycling conditions used in qPCR analyses in Cascade and Duplex (C+D), Revolution (REV), Sixteen 90 (16-90) PrimoMaxx (PM), Cutless (CL), Trimmit (TRIM) and non-treated control (NTC) soil samples collected 4cm from soil surface.

Target group	Gene	Amplicon length (bp)	Primers Name	Primers Sequence	Thermocycling conditions	References
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 3.3. Abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in the top 10 cm in response to application of three wetting agents surface applied using a backpack sprayer on research greens at the UGA Griffin Campus in Griffin, Georgia. All samples were taken a day after application.

		AOB (log copies g^{-1})			AOA (log copies g^{-1})		
	Sampling date	May 31, 2018	July 2, 2018	May 7, 2019	May 31, 2018	July 2, 2018	May 7, 2019
	# of applications	1	3	6	1	3	6
Treatment	Days since initial application	1	33	342	1	33	342
NTC		4.82a	4.62a	4.11a	4.17a	3.89a	3.47a
C+D		4.60a	4.41a	3.90a	3.92a	3.64a	3.22a
REV		4.81a	4.60a	4.10a	3.73a	3.45a	3.03a
16-90		4.45a	4.24a	3.74a	3.71a	3.43a	3.01a
p value		0.1663	0.1678	0.1713	0.0527	0.0532	0.0537

C+D = Cascade
Plus/Duplex REV = Revolution
16-90 = Sixteen 90
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 3.4. Abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in response to application of three plant growth regulators surface applied using a backpack sprayer at Rivermont Golf Club in Rivermont, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

		AOB (log copies g^{-1})		AOA (log copies g^{-1})	
	Sampling date	June 27, 2018	June 17, 2019	June 27, 2018	June 17, 2019
	# of applications	1	7	1	7
Treatment	Days since initial application	1	356	1	356
NTC		6.42a	5.94ba	4.12a	3.75a
PM		6.37a	5.88a	4.04a	3.65a
CT		6.33a	5.88a	3.97a	3.58a
TRIM		6.19a	5.79a	3.87a	3.49a
p value		0.1697	0.5498	0.3955	0.3790

PM = Primo Maxx CT = Cutless TRIM = Trimmit

Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within

Table 3.5. Soil respiration in response to application of three wetting agents surface applied using a backpack sprayer on research greens at the UGA Griffin Campus in Griffin, Georgia. All samples were collected from the top 10 cm a day after treatment

applications.

Treatment	Mean soil respiration (mg CO_2 - $g^{-1}'d^{-1}$)			
	Sampling time	May 31, 2018	July 2, 2018	May 7, 2019
	# of applications	1	3	6
	Days since initial application	1	33	342
NTC		1.92a	2.06a	1.81a
C+D		1.87a	2.01a	1.87a
REV		1.92a	2.00a	1.82a
16-90		1.87a	1.95a	1.80a
p value		0.834	0.2027	0.2245

C+D = Cascade Plus/Duplex; NTC = non-treated control
REV = Revolution
16-90 = Sixteen 90
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 3.6. Soil respiration in response to application of three plant growth regulators surface applied using a backpack sprayer at Rivermont Golf Club in Rivermont, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

Treatment	Mean soil respiration (mg CO_2 - g-1'h-1)		
	Sampling time	June 27, 2018	June 17, 2019
	# of applications	1	7
	Days since initial application	1	356
NTC		2.27a	1.85a
PM		1.93a	1.80a
CT		2.40a	1.92a
TRIM		2.23a	1.87a
p value		0.2782	0.6341

PM = Primo Maxx
CT = Cutless
TRIM = Trimmit
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 3.7. Phosphatase and urease activities in the top 10 cm in response to application of three wetting agents surface applied using a backpack sprayer on research greens at the UGA Griffin Campus in Griffin, Georgia. All samples were taken a day after application.

Treatment		Mean phosphatase activity (µmol P evolved g^{-1} h^{-1})			Mean urease activity (µmol NH3 evolved g^{-1} h^{-1})		
	Sampling date	May 31, 2018	July 2, 2018	May 7, 2019	May 31, 2018	July 2, 2018	May 7, 2019
	# of applications	1	3	6	1	3	6
	Days since initial application	1	33	342	1	33	342
NTC		1.47b	1.26a	1.42a	3.87a	3.12ab	2.60b
C+D		1.62ab	1.32a	1.60a	2.43c	2.60b	3.36ab
REV		1.99a	1.36a	1.81a	3.42ab	3.90a	4.24a
16-90		1.83ab	1.37a	1.74a	2.71bc	3.23ab	3.31ab
p value		0.0457	0.9258	0.5917	0.0010	0.0170	0.0410

C+D = Cascade
Plus/Duplex REV = Revolution
16-90 = Sixteen 90
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 3.8. Phosphatase and urease activities in the top 10 cm response to application of three plant growth regulators surface applied using a backpack sprayer at Rivermont Golf Club in Rivermont, Georgia. All samples were collected a day after treatment applications.

Treatment		Mean phosphatase activity (µmol P evolved g^{-1} h^{-1})		Mean urease activity (µmol NH3 evolved g^{-1} h^{-1})	
	Sampling date	June 27, 2018	June 17, 2019	June 27, 2018	June 17, 2019
	# of applications	1	7	1	7
	Days since initial application	1	356	1	356
NTC		1.62b	2.93b	34.54b	41.28a
PM		2.35ab	2.80b	41.66ab	35.12a
CT		3.10a	8.26a	42.42a	35.14a
TRIM		1.79b	8.81a	41.98ab	41.52a
p value		0.0103	<.0001	0.0273	0.1095

PM = Primo Maxx
CT = Cutless
TRIM = Trimmit
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 3.9. Turf quality in response to application of three wetting agents surface applied using a backpack sprayer on research greens at the UGA Griffin Campus in Griffin, Georgia.

	Turf Quality (% green cover)		
	Sampling time	June 2, 2018	May 19, 2019
Treatment	# of applications	1	3
	Days since initial application	1	33
NTC		97a	90ab
C+D		99a	92a
REV		100a	87ab
16-90		100a	87ab

C+D = Cascade
Plus/Duplex REV = Revolution
16-90 = Sixteen 90
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 3.10. Turf quality in response to application of three plant growth regulators surface applied using a backpack sprayer at Rivermont Golf Club in Rivermont, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

Treatment	Turf Quality (% green cover)		
	Sampling time	July 10, 2018	October 29, 2019
	# of applications	1	7
	Days since initial application	1	356
NTC		76a	88ab
PM		82a	93a
CT		78a	85b
TRIM		85a	91ab
p value			

PM = Primo Maxx CT = Cutless TRIM = Trimmit

Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Figure 3.1. Wetting Agent (WA) plot design at the UGA Griffin Campus in Griffin, Georgia.

105 Control	405 Sixteen 90	305 Revolution	205 Cascade/Duplex
304 Revolution	204 Cascade/Duplex	404 Sixteen 90	104 Control
103 Control	303 Revolution	203 Cascade/Duplex	403 Sixteen 90
202 Cascade/Duplex	402 Sixteen 90	302 Revolution	102 Control
101 Control	201 Cascade/Duplex	301 Revolution	401 Sixteen 90

Figure 3.2. Plant Growth Regulator (PGR) plot design at Rivermont Golf Club in John's Creek, Georgia.

205 Primo	305 Cutless	405 Trimmit	105 NTC
104 NTC	304 Cutless	204 Primo	404 Trimmit
403 Trimmit	303 Cutless	203 Primo	103 NTC
202 Primo	302 Cutless	102 NTC	402 Trimmit
201 Primo	301 Cutless	401 Trimmit	101 NTC

CHAPTER FOUR

EVALUATING THE IMPACT OF TWO BIOLOGICAL PRODUCTS ON TURF QUALITY AND SOIL BIOLOGICAL HEALTH[2]

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

ABSTRACT

Biological products often contain microbial inoculants and/or organic products and are marketed as being more sustainable than conventional products. This assumes that they are better in enhancing the beneficial services of soil microorganisms. However, there is lack of research on their efficacy. A field study was conducted at two locations in Georgia to evaluate the effects of two biological products (BP1 and BP2) on soil biological health and turf quality. A unique delivery system (A2G2) was used for applying the biological products directly in the root zone. Soil biological health was evaluated by determining microbial abundance and function. Turf quality was evaluated by calculating for normalized difference vegetation index from digital images of plots. Overall, there were statistically significant differences between the two products and how they were applied: but not between the biological products and non-treated control. Subsurface application of BP2 with A2G2 resulted in higher abundance of ammonia oxidizers, soil respiration and urease activity than surface or subsurface applications of BP1. This is most likely because BP2 had higher levels of carbon and nitrogen than BP1 and that when applied directly into the root zone it would increase microbial abundance and activity more so than BP1. None of the treatments significantly impacted turf quality, which did not show any significant correlation with the soil health parameters. Neither treatment contributed to the disease suppressive nature the soil as indicated by an inoculation study.

INTRODUCTION

Due to the need for aesthetics, the maintenance of golf courses entails extensive use of various inputs (e.g., fertilizers, pesticides, wetting agents, plant growth regulators, water). This makes it among the most expensive sector in agriculture (cost of input/acre). Reducing inputs is therefore important for the future of the golf course industry. This is particularly applicable to low and medium size clubs that have limited financial capacity (Beard, 1994). Reducing input is also important from the point of view of reducing the environmental footprint of golf courses.

Environmental concerns have led to the proliferation of biological products that are collectively called biostimulants (Calvo et al., 2014; du Jardin, 2012; Halpern et al., 2015). These products contain microorganisms (bugs in a jug) and/or organic products that are often marketed as being more sustainable and cheaper alternatives to current products that are commonly used in the golf course industry (du Jardin, 2012). This assumes that the biological products are better in stimulating the indigenous soil microorganisms that provide beneficial services (du Jardin, 2012). However, there is lack of research in evaluating how effective biological products are and how they affect the health of the turfgrass system and turf quality.

Biological products can boast the ability to improve soil health and turf quality through the promotion of N-fixation and mineralization, preventing and control pathogenic disease, and enhancing nutrient availability (du Jardin, 2012). In order to measure the efficacy of these claims we can study the soil biological health to essentially look at the effects of the products on the abundance and function of soil microorganisms. Specific biological indicators reflective of changes in soil can be used as measurement of soil biological health (Schloter, et al. 2003).

Microorganisms possess the ability to provide an integrated measure of soil health in a way that cannot be obtained through physical or chemical measures (Nielson and Winding, 2002). Measuring microbial abundance and function provides insight into the ways in which these products affect the soil health. Soil Respiration provides a generic measure of microbial activity (Schloter et al. 2003). Whereas enzymatic assays, including urease and phosphatase, provide more specific information on microbial cycling of N and P (Schloter et al. 2003).

One important consideration when evaluating biological products is the method of application. Products are commonly surface applied, leading to exposure of microorganisms contained in biological products to extreme climatic fluctuations (e.g., heat and UV exposure from sun). This exposure can reduce the survival and establishment of microbial inoculants in the soil. This can be minimized through subsurface application of the product. One way of achieving this is by using a unique tool such as Air2G2 as a delivery system directly to the root zone, which was originally designed to aerate the soil by blasting air below surface but has been modified to inject products. We examined if applying the products at the surface vs below surface with Air2G2 will make any difference in the performance of the products. We also compared the performance of products under two aerification techniques: hollow tine aerification, which is the conventional method and Air2G2, which is new.

The focus of this study was to determine the impacts of two biological products on soil biological health and turf quality and to determine how the performance of biological products is affected by methods of application and aerification.

METHODS AND MATERIALS

Study Setup and Treatment Application

Two field trials were established, one at Rivermont Golf Club in John's Creek, GA (June 2018) and one at Echelon Golf Club in Milton, GA (May 2019), to determine the effects of two biological products on soil biological health in warm and cool season turf grasses, respectively. Total rainfall in John's Creek (Tifgreen Bermudagrass) and Echelon (A1 Creeping Bentgrass) during the study periods were 79 cm and 57 cm respectively; average temperature ranged from 14°C to 27°C in John's Creek and 16°C to 28°C in Echelon (AEMN, 2019). A sensor and CR1000 datalogger (Campbell Scientific, Logan UT) was installed at the Rivermont location to capture soil temperature and moisture. Average soil temperature ranged from 23°C to 29°C in Echelon and 22°C to 29°C in Rivermont. Average volumetric soil moisture ranged from 22% to 32% at both locations (AEMN 2019).

The two biological products were KaPreRemeD8-NSL (BP1) and KaPreRemeD8-NSP (BP2) from Performance Nutrition (LidoChem, Inc., Hazlet, NJ). BP1 is described as a proprietary mixture including fulvic acid where as BP2 is described as a proprietary mixture containing *Saccharomyces cerevisiae*. The treatments were designed to apply the two biological products above or below surface coupled with or without aerification. The products were surface applied using a 15L backpack sprayer in which the products were applied twice at half the recommended rate to ensure adequate coverage and uniformity across plots. The Air2G2 machine was used for aeration and subsurface application of the products. The machine uses three 15-cm probes to laterally inject pressurized air into the top 10 cm of soil surface at a working width of 180 cm to provide aeration and ease compaction (GT AirInject, Inc. Jacksonville, FL). For subsurface injection, the machine was modified by fixing a three-

gallon tank to the top of the machine in order to allow product application directly to the root zone. Each plot receiving aerification from Air2G2 was injected with a total of 30 injection points in order to assure uniformity of coverage within each plot.

The combinations of mode of application and aeration resulted in the following seven treatments in total: 1) BP1 surface application without aerification, 2) BP2 surface application without aerification, 3) BP1 surface application and Air_2G_2 aerification, 4) BP2 surface application and Air_2G_2 aerification, 5) BP1 subsurface application with Air_2G_2 and 6) BP2 subsurface application with Air_2G_2 and 7) None-treated control (water) – No product or aerification. The idea behind subsurface application is to directly apply them to the root zone where the microbial inoculant in the product can be sheltered from the climatic variation in the surface. We introduced the aerification treatment to examine its impact on the product performance potentially through its effect on microbial activity.

The treatments were applied to plots that were arranged in a randomized complete block design including four replications of seven treatments at both locations. Each plot was 5.76 m^2 (2.4 x 2.4 m) in dimension. Treatments were applied based on the recommendation rates on the labels at 30 mL oz for BP1 and 562 mL for BP2 per 93 m^2 monthly. At the end of the study, the plots in Johns Creek had received fifteen treatments while the plots at Echelon had received seven treatments (Figure 4.1). The biological products were applied on the top of the standard turf management inputs. This included 1.36 kg N fertilizer in combination with .45 kg organic and synthetic urea and 0.91 kg inorganic ammonium sulfate, 0.23 kg of P in the form of soft rock phosphate, and 0.57 kg K using potassium sulfate. Greens also received spot treatment of Revolver for control of *Poa Annua* and Segway and Daconil for prevention of Pythium and leaf spot. Turf was maintained at

a 3.12- 2.67 mm mow height and received one-time aerification with half inch tines before initial study. The Control treatment received all standard inputs except for the biological products.

Sampling

Composite samples of 6 to 7 soil cores were collected from the research greens using a 127-mm soil probe to collect 10-cm soil columns from the greens. Soil samples were kept at 4°C until processed through a 2-mm sieve to remove plant material. Sieved samples were then used for abundance, soil respiration analyses, and enzyme assays. Five grams of each soil sample were placed in separate Ziploc bags and stored at -20°C for DNA extraction and quantitative polymerase chain reaction analysis. Dry soil weights were ascertained by placing all soil samples in an oven at 100°C for 24h and cooling in a desiccator for 2h. Moisture content of all samples was calculated and further used to calculate dry soil weight (g) equivalent of the amount used for analysis.

Sample Analysis

Biological soil health indicators that are reflective of the activity and abundance of soil microorganisms were monitored in the same manner as previously described in chapter three. Activity indicators included soil respiration and enzymes that mediate nitrogen and phosphorous transformations (urease and phosphatase). Enzyme activities were measured based on standard protocols in Tabatabai (1994). To quantify microbial abundance, DNA were extracted from all the samples with DNeasy PowerSoil kit (QIAGEN, Germantown, MD, USA). Quantitative

polymerase chain reaction was used to quantify the abundance of ammonia-oxidizing bacteria and archaea as described in Chapter three.

Turf quality was assessed by taking images of the plots with a digital camera and analyzing the images with the Assess 2.0 image analysis software (American Phytopathological Society) as percent green cover (ratio of green to total pixels). It provides an objective assessment of the overall turf quality and quantitative data for robust statistical analysis.

Inoculation Study

An inoculation study was initiated in July 2019 at both locations to test any changes in disease suppressive nature of the plots as a result of the use of the products. Among the common claims associated with biological products (from the manufacturers) is that they improve disease suppressive nature of the soil through their impact on soil microbial activity. To carry out the test, the top corner of each plot was inoculated with the pathogens that cause dollar spot (*Sclerotinia homoeocarpa*) and leaf spot (*Bipolaris sorokiniana*) in Echelon and Rivermont, respectively. The inocula were obtained from samples of greens containing disease on the Griffin Campus. Inocula were prepared by growing them in a medium that is described in Steketee (2016). Plots were rated visually based on the National Turfgrass Evaluation Program 1-9 rating scale to determine overall turf quality and percent disease.

Statistical Analysis

The data were summarized into descriptive statistics (e.g., mean and standard errors). Analysis of variance was carried out to test the statistical significance of the effects of the biological products and their mode of application on turf quality and indicators of soil health

at $\alpha = 0.05$ in JMP 14. Multivariate analysis using pairwise comparisons was to determine the relationship between soil health parameters and turf quality. Tukey's honest significant difference (HSD) test was used to conduct post hoc analyses to identify significant relationships among treatments within all models.

RESULTS AND DISCUSSION

Microbial Abundance

AOB ranged from 2.93-5.69 log copies g^{-1} in the Rivermont trial and 3.23-5.12 log copies g^{-1} in the Echelon trial. AOA ranged from 3.00-4.72 log copies g^{-1} and 3.20-5.34 log copies g^{-1} in the Rivermont and Echelon trials, respectively (Table 4.2 and 4.3). There were significant treatment effects on the abundance of ammonia-oxidizers in both trials. In the Rivermont trial, BP2 sub-surface A2G2 had a higher abundance of AOB and AOA as compared to the other treatments but not NTC. BP2 sub-surface A2G2 resulted in significantly higher AOB abundance than BP2 surface applied without aeration and BP1 sub A2G2 for the initial and last sampling times, respectively. Similarly, BP2 sub-surface A2G2 resulted in higher AOA abundance than BP1 surface w/A2G2 and BP2 surface after single application and BP1 sub A2G2 after five applications. This significant difference could be a result of the product (BP2) being placed directly into the root zone through use of the Air2G2 machine. Moreover, BP2 has a higher percent of N than BP1 and when applied directly to the root zone it could increase the abundance of ammonia oxidizers. We did not see as much a difference between the surface applications most likely because they were not being directly applied to the root zone and that the surface application might have exposed the constituents of the products (including microbial inoculants) to extreme climatic fluctuations.

In Echelon, NTC consistently had significantly higher abundance of both AOB and AOA than all the other treatments, which were not significantly different from each other throughout the study period (Table 4.3). In this case the products appear to have negatively impacted the abundance of ammonia oxidizers. A plausible reason for this could that the products might have negatively interacted with the other management inputs at this site (which was different from Rivermont), causing an overall decline in microbial abundance.

Soil Respiration

In Rivermont, soil respiration ranged from 2.12 - 1.82 mg CO_2 $g^{-1} \cdot d^{-1}$ and 2.10 - 1.79 mg CO_2 - $g^{-1} \cdot d^{-1}$ in Echelon (Table 4.4 and 4.5, respectively). There was significant treatment effect in the initial sampling time at Rivermont, where BP2 Surface without aerification resulted in higher soil respiration than BP1 sub A2G2 and NTC. In comparing the two products, BP2 has a higher percent of carbon than BP1 (Table 4.1). After the initial sampling time, there were no noted significant treatment effects in Rivermont. The initial increase in respiration is an indication of the temporary impact of the product in stimulating microbial activity as a carbon source.

In Echelon, the only significant treatment effect was seen during the last sampling date, after the treatments were applied nine times. The BP2 Sub-surface A2G2 treatment produced higher rates than the BP1 surface with A2G2 aeration treatment. Given the carbon content in BP2, it makes sense that it would produce a higher respiration rate. Another plausible explanation is that foliar (surface) applications can lead to products staying within the thatch layer, upon sampling and sieving this layer is removed before lab analysis. This is supported by findings of Mueller and Kussow (2005) that reported removing the thatch layer limited the detection of biostimulant influences on microbial activity. Despite being established a year apart both Rivermont and

Echelon produced similar results in soil respiration in that BP2 was the treatment that resulted in higher respiration rate.

Phosphatase

Phosphatase activity ranged from 0.703-1.527 µmol P g^{-1} h^{-1} in Rivermont and 0.079-0.527 µmol P g^{-1} h^{-1} in Echelon Tables 4.6 and 4.7, respectively. In Rivermont, the NTC was significantly different as compared to BP1 sub-surface application, with the control having a higher rate of phosphatase activity. It appears that the product caused a temporary decrease in activity as this trend was not seen throughout the duration of the study. In Echelon, there was no significant treatment effect on phosphatase activity.

One major reason for not observing significant impacts can be in part due to the fact that we were testing these products on healthy systems. In this case, both trial areas receive constant input of water and nutrients, the fact that they are not deprived of anything makes it harder to note any significance in that the system has everything it needs, and the addition of supplements makes no marked difference (Hopkins, 2019).

Urease

Soil urease activity ranged from 4.68 - 17.75 µmol NH_3 evolved g^{-1} h^{-1} and 0.403-5.52 µmol NH_3 evolved g^{-1} h^{-1} in Rivermont and Echelon, respectively (Tables 4.6 and 4.7). There were no significant treatment effects on urease activity in Rivermont. However, urease activity increased over time. In Echelon, BP2 Sub A2G2 resulted in significantly higher urease activity than all other treatment applications excluding NTC and BP1 Sub A2G2. The same trend is seen in the final sampling date where BP2 Sub A2G2 had significantly higher urease activity compared

against all other treatments. This is most likely due to the fact that BP2 contains 30% N compared to the much lower amount of N present in BP1 (Table 4.1). The application of this product with a high N content directly to the root zone might have caused the increase in urease activity.

Comparing the two sites, Rivermont had much higher rates of urease activity than Echelon. This could most likely be due to the level of inputs and differing management of the two greens and the duration of the separate studies. Higher urease activity could be attributed to the accumulation of substrate from the continued application of the product. This observation agrees with findings of Huang et al. (1992) which reported that urease activity is substrate dependent, increasing with substrate concentration to reach a maximum.

Inoculation Study

The initial inoculation at Rivermont resulted in no leaf spot appearing on any plots, mainly due to high temperatures and lack of moisture. In Echelon, dollar spot appeared on all of the plots, but there were no differences among the treatments. Plots were inoculated again at the end of September and beginning of October. The second inoculation resulted in similar fashion with all plots showing leaf spot in Rivermont and all plots showing dollar spot in Echelon.

This can provide insight into the validity of the products. Both products claim to be disease suppressants, however, all plots showed signs of disease. One major issue is that most of these microbial inoculants take years to establish in the soil (Trabelsi and Mhamdi, 2013). Rivermont received a full year of applications, whereas Echelon only received three total applications. This can also be due to the difficulty in identifying modes of action in the different biological products on the market as noted by Yakhin et al. (2017).

Turf Quality

There were no significant treatment effects on turf quality at either location (Tables 4.8 and 4.9, respectively). The only noted difference is the drastic decline in turf quality at the last sampling date in Rivermont. This is due in part to the inoculation study as these measurements were taken the same day of inoculation rates in Rivermont. There were also no noted significant correlations between the turf quality and soil biological health parameters.

SUMMARY AND CONCLUSIONS

In this study, we evaluated the impact of two biological products (BP1 and BP2) on soil biological health and turf quality parameters in field studies in two golf courses. The biological products were applied in two different ways, surface and subsurface. The subsurface application was carried out by a uniquely designed machine (A2G2) that directly injects the products into the root zone. The products were also applied in combination with or without aerification. Overall, the statistically significant differences were not between the products and the non-treated control (NTC) rather between the two products and how they were applied. Subsurface application of BP2 with A2G2 resulted in higher abundance of ammonia oxidizers, soil respiration and urease activity than surface or subsurface applications of BP1. This could be because BP2 had higher levels of carbon and nitrogen than BP1 and that when applied below surface directly into the root zone, it would increase microbial abundance and activity more so than BP1. None of the treatments significantly impacted turf quality, which did not show any significant correlation with the soil health parameters. The treatments did not either contribute to the disease suppressive nature of the soil as indicated by the inoculation study. Overall, the products did not seem to have negatively impacted soil biological health. They also were not beneficial in

improving the turf quality as compared to the NTC.

Future research would require the addition of a positive control alongside the negative. In this sense we would be able to ascertain the viability of these products and eliminate background effects of fertilizers, pesticides, or any other added inputs.

REFERENCES

Anderson, T. H., "Microbial Eco-Physiological Indicators to Assess Soil Quality." *Agriculture, Ecosystems and Environment*, vol. 98, no. 1–3, 2003, pp. 285–93, doi:10.1016/S0167-8809(03)00088-4.

Barrios, E. "Soil Biota, Ecosystem Services and Land Productivity." *Ecological Economics*, vol. 64, no. 2, 2007, pp. 269–85, doi:10.1016/j.ecolecon.2007.03.004.

Bartlett, M. D., et al. "Interactions between Microbial Community Structure and the Soil Environment Found on Golf Courses." *Soil Biology and Biochemistry*, vol. 39, no. 7, 2007, pp. 1533–41

Barton, L., and T. D. Colmer. "Ameliorating Water Repellency under Turfgrass of Contrasting Soil Organic Matter Content: Effect of Wetting Agent Formulation and Application Frequency." *Agricultural Water Management*, vol. 99, no. 1, Elsevier B.V., 2011, pp. 1–7, doi:10.1016/j.agwat.2011.08.009.

Beard, J. B., and R. L. Green. "The Role of Turfgrasses in Environmental Protection and Their Benefits to Humans." *Journal of Environmental Quality*, vol. 23, no. 3, 1994, pp. 452–60, doi:10.2134/jeq1994.00472425002300030007x.

Bonanomi, G., et al. "Organic Amendments, Beneficial Microbes, and Soil Microbiota: Toward a Unified Framework for Disease Suppression." *Annual Review of*

Phytopathology, vol. 56, no. 1, 2018, pp. 1–20, doi:10.1146/annurev-phyto-080615-100046.

Burns, R. G., et al. "Soil Enzymes in a Changing Environment: Current Knowledge and Future Directions." *Soil Biology and Biochemistry*, vol. 58, Elsevier Ltd, 2013, pp. 216–34, doi:10.1016/j.soilbio.2012.11.009.

Chen, H., et al. "The Soil Microbial Community of Turf: Linear and Nonlinear Changes of Taxa and N-Cycling Gene Abundances over a Century-Long Turf Development." *FEMS Microbiology Ecology*, vol. 95, no. 2, Oxford University Press, 2019, pp. 1–14, doi:10.1093/femsec/fiy224.

Chen, H., et al. "The Soil Microbial Community of Turf: Linear and Nonlinear Changes of Taxa and N-Cycling Gene Abundances over a Century-Long Turf Development." *FEMS Microbiology Ecology*, vol. 95, no. 2, Oxford University Press, 2019, pp. 1–14, doi:10.1093/femsec/fiy224.

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

Cotton, S. "Wetting Agents: What Are They and How Do They Work?" *American Scientist*, vol. 106, no. 3, 2018, p. 138, doi:10.1511/2018.106.3.138.

Crecchio, C., et al. *Beneficial Soil Microbiome for Sustainable Agriculture Production*. no. September 2018, pp. 443–81, doi:10.1007/978-3-319-94232-2_9.

Cutler, N. R., et al. "Soil Health and Sustainability." *Life Sciences*, vol. 62, no. 16, 1998, pp. 1433–41, doi:10.1016/S0024-3205(98)00082-4.

Das, S. K., and A. V., "Soil Enzymology." *Soil Enzymology*, vol. 22, 2010, pp. 275–85, doi:10.1007/978-3-642-14225-3.

Dell, E. A., et al. “Heat Stress and N Fertilization Affect Soil Microbial and Enzyme Activities in the Creeping Bentgrass (Agrostis Stolonifera L.) Rhizosphere.” *Applied Soil Ecology*, vol. 56, 2012, pp. 19–26, doi:10.1016/j.apsoil.2012.02.002.

Diera, AA, Raymer, PL, Martinez-Espinoza, AD, Bauske, E, Habteselassie, MY. Evaluating the impact of turf-care products on soil biological health. *J. Environ. Qual*. 2020; 49: 858– 868. https://doi.org/10.1002/jeq2.20080

du Jardin, P. “Plant Biostimulants: Definition, Concept, Main Categories and Regulation.” *Scientia Horticulturae*, vol. 196, Elsevier B.V., 2015, pp. 3–14, doi:10.1016/j.scienta.2015.09.021.

Fierer, N., et al. “Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays.” *Applied and Environmental Microbiology*, vol. 71, no. 7, 2005, pp. 4117–20, doi:10.1128/AEM.71.7.4117-4120.2005.

Fry, J., et al. *Reducing Fungicide Use on Creeping Bentgrass Greens*. no. July 2006, pp. 77–81.

González-vila, F. J., and A. S. Ball. *Soil Health — a New Challenge for Microbiologists And*. 2013, pp. 13–21.

Harman, G. E., et al. “Non-Target Effects of Fungicide Applications on Microbial Populations of Putting Greens.” *USGA Green Sec. Rec.*, vol. 44, no. 4, 2006, pp. 9–12.

Hill, G. T., et al. “Methods for Assessing the Composition and Diversity of Soil Microbial Communities.” *Applied Soil Ecology*, vol. 15, no. 1, 2000, pp. 25–36, doi:10.1016/S0929- 1393(00)00069-X.

Kennedy, A. C., and R. I. Papendick. *Microbial Characteristics of Soil Quality*. no. June, 1995, pp. 243–48.

Kokkoris, V., et al. “Site Specificity in Establishment of a Commercial Arbuscular Mycorrhizal Fungal Inoculant.” *Science of the Total Environment*, vol. 660, Elsevier B.V., 2019, pp. 1135–43, doi:10.1016/j.scitotenv.2019.01.100.

Lee, J., et al. “Dollar Spot and Brown Patch Incidence in Creeping Bentgrass as Affected by Acibenzolar-S-Methyl and Biostimulants.” *HortScience*, vol. 38, no. 6, 2003, pp. 1223–26, doi:10.21273/hortsci.38.6.1223.

Liu, Y., et al. “Microbial and Soil Properties in Bentgrass Putting Greens: Impacts of Nitrogen Fertilization Rates.” *Geoderma*, vol. 162, no. 1–2, Elsevier B.V., 2011, pp. 215–21, doi:10.1016/j.geoderma.2011.02.009.

Lo, C. T., et al. “Improved Biocontrol Efficacy of Trichoderma Harzianum 1295-22 for Foliar Phases of Turf Diseases by Use of Spray Applications.” *Plant Disease*, vol. 81, no. 10, 1997, pp. 1132–38, doi:10.1094/PDIS.1997.81.10.1132.

Milesi, C., et al. “Mapping and Modeling the Biogeochemical Cycling of Turf Grasses in the United States.” *Environmental Management*, vol. 36, no. 3, 2005, pp. 426–38, doi:10.1007/s00267-004-0316-2.

Moebius-Clune, B.N, et al. *Comprehensive Assessment of Soil Health*. 2016, http://www.nysaes.cornell.edu.

Mueller, S. R., and W. R. Kussow. “Biostimulant Influences on Turfgrass Microbial Communities and Creeping Bentgrass Putting Green Quality.” *HortScience*, vol. 40, no. 6, 2005, pp. 1904–10, doi:10.21273/hortsci.40.6.1904.

Mukerji, K. G. “Soil Microbes.” *Techniques in Mycorrhizal Studies*, vol. 50, 2002, pp. 7–13, doi:10.1007/978-94-017-3209-3_2.

Mundepi, A., et al. “Ammonia Oxidizers as Biological Health Indicators of Elevated Zn and

Cu in Poultry Litter Amended Soil." *Water, Air, and Soil Pollution*, vol. 230, no. 10, Water, Air, & Soil Pollution, 2019, doi:10.1007/s11270-019-4283-x.

Nannipieri, P., et al. "Soil Enzyme Activity: A Brief History and Biochemistry as a Basis for Appropriate Interpretations and Meta-Analysis." *Biology and Fertility of Soils*, vol. 54, no. 1, Biology and Fertility of Soils, 2018, pp. 11–19, doi:10.1007/s00374-017-1245-6.

Nielsen, M. N., and A. Winding. "Microorganisms as Indicators of Soil Health." *Neri*, vol. NERI Techn, no. 388, 2002, http://www.dmu.dk.

Sahrawat, K. L. (1984). Effects of temperature and moisture on urease activity in semi-arid tropical soils. Plant and Soil, 78(3), 401-408.

Saporta, R., et al. "A Method for a Fast Evaluation of the Biostimulant Potential of Different Natural Extracts for Promoting Growth or Tolerance against Abiotic Stress." *Agronomy*, vol. 9, no. 3, 2019, doi:10.3390/agronomy9030143.

Schlatter, D., et al. "Disease Suppressive Soils: New Insights from the Soil Microbiome." *Phytopathology*, vol. 107, no. 11, 2017, pp. 1284–97, doi:10.1094/PHYTO-03-17-0111- RVW.

Schloter, M., et al. "Indicators for Evaluating Soil Quality." *Agriculture, Ecosystems and Environment*, vol. 98, no. 1–3, 2003, pp. 255–62, doi:10.1016/S0167-8809(03)00085-9.

Sher, Y., et al. "Ammonia Transformations and Abundance of Ammonia Oxidizers in a Clay Soil Underlying a Manure Pond." *FEMS Microbiology Ecology*, vol. 81, no. 1, 2012, pp. 145–55, doi:10.1111/j.1574-6941.2012.01347.x.

Shi, W., et al. "Soil Microbial Community Composition and Function in Turfgrass Ecosystems." *Bioremediation, Biodiversity and Bioavailability*, vol. 1, no. 1, 2007, pp.

72–77.

Shi, W., et al. “Soil Enzyme Activities and Organic Matter Composition in a Turfgrass Chronosequence.” *Plant and Soil*, vol. 288, no. 1–2, 2006, pp. 285–96, doi:10.1007/s11104-006-9116-1.

Shi, W., et al. “Soil Microbial Biomass, Activity and Nitrogen Transformations in a Turfgrass Chronosequence.” *Soil Biology and Biochemistry*, vol. 38, no. 2, 2006, pp. 311–19, doi:10.1016/j.soilbio.2005.05.008.

Shimada, M. “Agarose Gel Electrophoresis.” *Tanpakushitsu Kakusan Koso. Protein, Nucleic Acid, Enzyme*, vol. 41, no. 5, 1996, pp. 477–79.

Song, Enzhan, et al. “Influence of Repeated Application of Wetting Agents on Soil Water Repellency and Microbial Community.” *Sustainability (Switzerland)*, vol. 11, no. 16, 2019, pp. 1–13, doi:10.3390/su11164505.

Song, E., et al. “Influence of Repeated Application of Wetting Agents on Soil Water Repellency and Microbial Community.” *Sustainability (Switzerland)*, vol. 11, no. 16, 2019, pp. 1–13, doi:10.3390/su11164505.

Steketee, C. J., et al. “Effects of Genotype and Isolate on Expression of Dollar Spot in Seashore Paspalum.” *HortScience*, vol. 51, no. 1, 2016, pp. 67–73, doi:10.21273/hortsci.51.1.67.

Strandberg, M., et al. “Priorities for Sustainable Turfgrass Management: A Research and Industry Perspective.” *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, vol. 62, no. SUPPL.1, 2012, pp. 3–9, doi:10.1080/09064710.2012.682163.

Taliaferro, C. M. “Diversity and Vulnerability of Bermuda Turfgrass Species.” *Crop Science*, vol. 35, no. 2, 1995, p. 327,

doi:10.2135/cropsci1995.0011183x003500020006x.

Thompson, G. L., and J. Kao-Kniffin. “Diversity Enhances NPP, N Retention, and Soil Microbial Diversity in Experimental Urban Grassland Assemblages.” *PLoS ONE*, vol. 11, no. 5, 2016, pp. 1–17, doi:10.1371/journal.pone.0155986.

Thompson, G. L., and J. Kao-Kniffin. “Urban Grassland Management Implications for Soil c and n Dynamics: A Microbial Perspective.” *Frontiers in Ecology and Evolution*, vol. 7, no. AUG, 2019, pp. 1–15, doi:10.3389/fevo.2019.00315.

Trabelsi, D, and R Mhamdi. “Microbial Inoculants and Their Impact on Soil Microbial Communities: A Review.” *BioMed Research International*, vol. 2013, 2013, doi:10.1155/2013/863240.

Trognitz, F., et al. “The Role of Plant-Microbiome Interactions in Weed Establishment and Control.” *FEMS Microbiology Ecology*, vol. 92, no. 10, 2016, pp. 1–15, doi:10.1093/femsec/fiw138.

Utobo, E. B. “Soil Enzymes as Bioindicators of Soil Ecosystem Status.” *Applied Ecology and Environmental Research*, vol. 13, no. 1, 2014, pp. 85–97, doi:10.15666/aeer/1301.

Van Bruggen, A. H. C., and A. M. Semenov. “In Search of Biological Indicators for Soil Health and Disease Suppression.” *Applied Soil Ecology*, vol. 15, no. 1, 2000, pp. 13–24, doi:10.1016/S0929-1393(00)00068-8.

Van Der Heijden, M G. A., et al. “The Unseen Majority: Soil Microbes as Drivers of Plant Diversity and Productivity in Terrestrial Ecosystems.” *Ecology Letters*, vol. 11, no. 3, 2008, pp. 296–310, doi:10.1111/j.1461-0248.2007.01139.x.

Wyngaard, N, et al. “Legacy Effect of Fertilization and Tillage Systems on Nitrogen

Mineralization and Microbial Communities." *Soil Science Society of America Journal*, vol. 80, no. 5, 2016, pp. 1262–71, doi:10.2136/sssaj2016.03.0070.

Yakhin, O. I., et al. "Biostimulants in Plant Science: A Global Perspective." *Frontiers in Plant Science*, vol. 7, 2017, doi:10.3389/fpls.2016.02049.

Yao, H, et al. "Soil Microbial Community Structure and Diversity in a Turfgrass Chronosequence: Land-Use Change versus Turfgrass Management." *Applied Soil Ecology*, vol. 34, no. 2–3, 2006, pp. 209–18, doi:10.1016/j.apsoil.2006.01.009.

Zontek, B. Y. Stanley J. *Understanding the Different Wetting Agent Chemistries*. Green Record, vol. 50 2012, pp. 1–6.

Table 4.1. Description of the two biological products (BP) used in the study.

Treatment	Product Type	Trade Name	Manufacturer	Active Ingredient (a.i.)	Rate of Application -L a.i. ha^{-1}-	Trial Location	% C & N
BP1	Biological Product	KaPre RemeD8 NSL	Lido Chemical	N/A	0.53	Rivermont/ Echelon Putting Greens	0.7, 0.08
BP2		KaPre RemeD8 NSP	Lido Chemical	N/A	0.03		30, 2.3

Table 4.2. Abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in response to application of the two biological products applied on surface or subsurface using the Air2G2 machine in turf green in combination with aerification treatments at the Rivermont Golf Club in Johns Creek, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

		AOB (log copies g^{-1})			AOA (log copies g^{-1})		
	Sampling date	June 13, 2018	October 25, 2018	June 11, 2019	June 13, 2018	October 25, 2018	June 11, 2019
	# of applications	1	5	11	1	5	11
Treatment	Days since initial application	1	135	364	1	135	364
NTC		4.33ab	5.08a	5.04ab	3.68ab	4.34ab	4.39a
BP1 Surface		4.23ab	5.17a	5.12a	3.76ab	4.41ab	4.48a
BP2 Surface		2.93b	4.92a	4.93ab	3.30b	4.22ab	3.00a
BP1 surface w/A2G2		3.73ab	4.96a	5.03ab	3.38b	4.32ab	4.24a
BP2 surface w/A2G2		4.31ab	5.08a	4.93ab	3.61ab	4.23ab	4.13a
BP1 Sub A2G2		4.37ab	4.87a	4.85b	3.71ab	4.15b	4.33a
BP2 Sub A2G2		4.97a	5.69a	5.16a	4.10a	4.45a	4.72a
p-value		0.0065	0.2769	0.0082	0.0036	0.0196	0.1207

BP1 = KaPre RemeD8 NSL
BP2 = KaPreRemeD8 NSP
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 4.3. Abundance of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in response to application of the two biological products applied on surface or subsurface using the Air2G2 machine in turf green in combination with aerification treatments at the Echelon Golf Club in Milton, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

		AOB (log copies g^{-1})			AOA (log copies g^{-1})		
	Sampling date	May 29, 2019	October 7, 2019	December 18, 2019	May 29, 2019	October 7, 2019	December 18, 2019
	# of applications	2	7	9	2	7	9
Treatment	Days since initial application	1	132	204	1	132	204
NTC		4.68a	4.63a	5.12a	4.70a	5.03a	5.34a
BP1 Surface		3.74b	3.58b	4.07b	3.83b	3.98b	3.20b
BP2 Surface		3.69b	3.48b	3.96b	3.81b	3.87b	4.18b
BP1 surface w/ A2G2		3.79b	3.63b	4.12b	3.88b	4.03b	4.34b
BP2 surface w/ A2G2		3.68b	3.53b	4.01b	3.78b	3.93b	4.23b
BP1 Sub A2G2		3.61b	3.41b	3.89b	3.73b	3.81b	4.11b
BP2 Sub A2G2		3.50b	3.23b	3.74b	3.64b	3.65b	3.96b
p-value		0.0003	0.0009	0.0009	0.0003	0.0009	0.0178

BPI = KaPre RemeD8 NSL
BP2 = KaPreRemeD8 NSP
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 4.4. Soil respiration in response to application of the two biological products applied on surface or subsurface using the Air2G2 machine in turf green in combination with aerification treatments at the Rivermont Golf Club in Johns Creek, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

	Mean soil respiration (mg CO_2 $g^{-1}soil \cdot d^{-1}$)		
Sampling time	June 13, 2018	October 25, 2018	June 11, 2019
# of applications	1	5	11
Days since initial application	1	135	364
Treatment			
NTC	1.87bc	1.95a	2.01a
BP1 Surface	1.86bc	1.82a	2.04a
BP2 Surface	2.05a	2.12a	2.05a
BP1 w/ A2G2	1.98ab	1.90a	1.96a
BP2 w/ A2G2	1.97abc	1.88a	1.96a
BP1 Sub A2G2	1.83c	1.90a	1.99a
BP2 Sub A2G2	1.95abc	1.93a	2.03a
p-value	0.0016	0.4044	0.6002

BP1 = KaPreRemeD8 NSL
BP2 = KaPreRemeD8 NSP
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 4.5. Soil respiration in response to application of the two biological products applied on surface or subsurface using the Air2G2 machine in turf green in combination with aerification treatments at the Echelon Golf Club in Milton, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

Treatment	Mean soil respiration (mg CO_2 g^{-1}soil·d^{-1})			
	Sampling time	May 29, 2019	October 7, 2019	December 18, 2019
	# of applications	2	7	9
	Days since initial application	1	132	204
NTC		1.99a	1.82a	1.91ab
BP1 Surface		1.97a	1.86a	1.88ab
BP2 Surface		2.10a	1.90a	1.79b
BP1 w/ A2G2		1.92a	1.90a	1.81ab
BP2 w/ A2G2		1.99a	1.92a	1.89ab
BP1 Sub A2G2		1.94a	1.89a	1.82ab
BP2 Sub A2G2		1.95a	1.87a	1.92a
p-value		0.5175	0.0543	0.0119

BPI = KaPre RemeD8 NSL
BP2 = KaPreRemeD8 NSP
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 4.6. Phosphatase and urease activities in response to application of the two biological products applied on surface or subsurface using the Air2G2 machine in turf green in combination with aerification treatments at the Rivermont Golf Club in Johns Creek, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

		Mean phosphatase activity (µmol P g^{-1} h^{-1})			Mean urease activity (µmol NH3 g^{-1} h^{-1})		
	Sampling date	June 13, 2018	October 25, 2018	June 11, 2019	June 13, 2018	October 25, 2018	June 11, 2019
	# of applications	1	5	11	1	5	11
Treatment	Days since initial application	1	135	364	1	135	364
NTC		1.527a	0.800a	0.868a	5.75a	10.25a	17.75a
BP1 Surface		1.048ab	1.100a	0.985a	4.68a	7.50a	13.0a
BP2 Surface		1.398ab	1.250a	1.170a	6.45a	8.25a	14.0a
BP1 surface w/A2G2		1.198ab	1.450a	0.800a	7.28a	8.25a	16.75a
BP2 surface w/A2G2		1.158ab	1.375a	0.703a	4.88a	6.50a	12.25a
BP1 Sub A2G2		0.798b	0.950a	0.738a	6.35a	7.75a	19.5a
BP2 Sub A2G2		0.980ab	1.375a	0.990a	4.85a	8.50a	14.75a
p-value		0.0344	0.2597	0.6358	0.7652	0.5025	0.3089

BPI = KaPre RemeD8 NSL
BP2 = KaPre RemeD8 NSP
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 4.7 Phosphatase and urease activities in response to application of the two biological products applied on surface or subsurface using the Air2G2 machine in turf green in combination with aerification treatments at the Echelon Golf Club in Milton, Georgia. All samples were collected from the top 10 cm a day after treatment applications.

		Mean phosphatase activity (µmol P g^{-1} h^{-1})			Mean urease activity (µmol NH3 g^{-1} h^{-1})		
	Sampling date	May 29, 2019	October 7, 2019	December 18, 2019	May 29, 2019	October 7, 2019	December 18, 2019
	# of applications	2	7	9	2	7	9
Treatment	Days since initial application	1	132	204	1	132	204
NTC		0.234a	0.190a	0.497a	0.818abc	5.52a	1.22bc
BP1 Surface		0.307a	0.227a	0.499a	0.415bc	5.48a	1.32bc
BP2 Surface		0.358a	0.139a	0.501a	0.408bc	5.24a	0.870cd
BP1 surface w/A2G2		0.454a	0.185a	0.510a	0.288c	5.78a	0.658d
BP2 surface w/A2G2		0.499a	0.165a	0.501a	0.403bc	5.02a	1.20bc
BP1 Sub A2G2		0.527a	0.079a	0.465a	0.865ab	4.97a	1.54b
BP2 Sub A2G2		0.393a	0.077a	0.462a	1.26a	5.28a	3.12a
p-value		0.2433	0.2222	0.0606	0.0001	0.2411	<0.0001

BPI = KaPre RemeD8 NSL
BP2 = KaPreRemeD8 NSP
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 4.8. Turf quality in response to application of the two biological products applied on surface or subsurface using the Air2G2 machine in turf green in combination with aerification treatments at the Rivermont Golf Club in Johns Creek, Georgia.

	Turf Quality (% green cover)			
Treatment	Sampling time	June 13, 2018	October 25, 2018	June 11, 2019
	# of applications	1	5	11
	Days since initial application	1	135	364
NTC		96a	96a	65a
BP1 Surface		95a	95a	59a
BP2 Surface		96a	96a	61a
BP1 w/ A2G2		95a	95a	58a
BP2 w/ A2G2		92a	92a	58a
BP1 Sub A2G2		93a	93a	65a
BP2 Sub A2G2		97a	97a	61a

BPI = KaPre RemeD8 NSL
BP2 = KaPreRemeD8 NSP
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Table 4.9. Turf quality in response to application of the two biological products applied on surface or subsurface using the Air2G2 machine in turf green in combination with aerification treatments at the Echelon Golf Club in Milton, Georgia.

Treatment	Turf Quality (% green cover)		
	Sampling time	July 10, 2018	October 29, 2019
	# of applications	2	7
	Days since initial application	1	132
NTC		96a	94a
BP1 Surface		93a	90a
BP2 Surface		90a	84a
BP1 w/ A2G2		90a	89a
BP2 w/ A2G2		95a	92a
BP1 Sub A2G2		90a	92a
BP2 Sub A2G2		90a	94a

BPI = KaPre RemeD8 NSL
BP2 = KaPreRemeD8 NSP
Values with different letter suffix are significantly different at p = 0.5. Comparison is valid within sampling date columns.

Figure 4.1. Biologicals (BP) plot design at both Echelon Golf Club and Rivermont Golf Club.

101 NTC	301 BP2 Surface	701 BP2 Sub A2G2	401 BP1 w/ A2G2	501 BP2 w/ A2G2	201 BP1 Surface	601 BP1 Sub A2G2
502 BP2 w/ A2G2	602 BP1 Sub A2G2	402 BP1 w/ A2G2	202 BP1 Surface	102 NTC	702 BP2 Sub A2G2	302 BP2 Surface
403 BP1 w/ A2G2	303 BP2 Surface	503 BP2 w/ A2G2	703 BP2 Sub A2G2	603 BP1 Sub A2G2	103 NTC	203 BP1 Surface
604 BP1 Sub A2G2	104 NTC	204 BP1 Surface	304 BP2 Surface	504 BP2 w/ A2G2	404 BP1 w/ A2G2	704 BP2 Sub A2G2

CHAPTER FIVE

SUMMARY AND CONCLUSIONS

In this study, the impacts wettings agents (WAs), plant growth regulators (PGRs) and biological products (BPs) were evaluated in a field study that lasted up to a year. The products were applied on experimental field plots in golf greens and fairways and their impact on turf quality and soil biological health was evaluated over time after single and multiple applications. Turf quality was evaluated by assessing the change in percent green cover. The soil biological health was assessed by measuring for soil health parameters that included microbial abundance for ammonia oxidizers, soil respiration and enzyme activities.

Overall, the WAs and PGRs did not significantly impact the abundance of ammonia oxidizers or soil respiration. The impact of the treatments on soil biological health was mainly reflected on soil enzyme activities. Revolution from WAs and Cutless and Trimmit from PGRs resulted in significant increase in phosphatase activity. Urease activity, on the other hand, was initially suppressed by WAs C+D and 16-19 while it was stimulated by Revolution after multiple applications. The impact of Cutless on urease activity was positive but shorted lived. The positive impacts of these treatments were probably because of their effect in improving soil moisture availability (WAs) and root growth (PGRs) that would in turn improve microbial activity as reflected by the enzyme activities. When negative impacts of some WAs were observed (i.e., on urease) they were temporary. Some WAs were observed to cause phytotoxicity on the turf and might have also negatively impacted microbial activity. None of the WAs improved turf quality as compared to the Control at both sampling times. The PGRs Primo Maxx and Trimmit, on the other hand, significantly improved turf quality after multiple applications.

In the biological study, the statistically significant differences were not between the

products and the non-treated control (NTC) rather between the two products and how they were applied. Subsurface application of BP2 with A2G2 resulted in higher abundance of ammonia oxidizers, soil respiration and urease activity than surface or subsurface applications of BP1. This could be because BP2 had higher levels of carbon and nitrogen than BP1 and that when applied below surface directly into the root zone it would increase microbial abundance and activity more so than BP1. None of the treatments significantly impacted turf quality, which did not show any significant correlation with the soil health parameters. The treatments did not either contribute to the disease suppressive nature of the soil as indicated by the inoculation study. Overall, the products did not seem to have negatively impacted soil biological health. They also were not beneficial in improving the turf quality as compared to the NTC.

It is important to note that this is a relatively short study time. A long-term study might provide a better insight on the implications of using these products on sustainability of the turfgrass system in the future.