

LABORATORY AND GREENHOUSE STUDIES OF IMPACT OF TURF CARE
PRODUCTS ON SOIL BIOLOGICAL HEALTH

by

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(Under the Direction of Mussie Y. Habteselassie)

ABSTRACT

Laboratory and greenhouse studies were conducted to investigate the impacts of selected wetting agents (WAs), plant growth regulators (PGRs), and biological products (BPs) on turf quality and soil biological health using soil respiration, phosphatase activity, and urease activity as indicators of soil biological health. Disease suppressive nature of soil following the application of these treatments was investigated by inoculating grass with Dollar spot (*Clarireedia* spp. formerly *Sclerotinia homoeocarpa*) pathogen. In the Laboratory study, among WAs, Sixteen 90 and Duplex enhanced soil respiration whiles Vivax and Magnus suppressed it. Urease activity was enhanced by Vivax, Fleet, Magnus and Sixteen 90. Phosphatase activity was enhanced by only Dispatch. Among PGRs, Anuew, Cutless, and Proxy enhanced soil respiration, phosphatase and urease activity respectively. BPs did not significantly impact the variables of this study. In the Greenhouse study, among WAs, Vivax, Fleet, Magnus, and Dispatch enhanced soil respiration whereas phosphatase and urease were not significantly impacted by WAs. In the Greenhouse study, among PGRs, Cutless, Trimmit, and Anuew

suppressed soil respiration. None of BPs significantly impacted the variables of this study in the Greenhouse. Turf quality was significantly improved by WAs, Revolution, Cascade, Pervade, and Sixteen 90, 7 weeks after treatment whiles Kapre Reme NSL, a BP also improved turf quality. Disease suppressive nature of soil was not significantly improved by any treatment.

INDEX WORDS: Wetting agent, plant growth regulator, biological products, Dollar spot, phosphatase activity, urease activity, soil respiration, disease suppressive nature, turf quality.

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DEDICATION

I dedicate this thesis to my mother and exceptional aunties. My mother, Mary Asromaa, has been of incredible help in coming this far. She has been my source of emotional strength in achieving this feat. My aunties, Edith Koranteng, Judith Koranteng, and Doris Appiah showed their love when it mattered most and without them, all this would not be possible.

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CHAPTER 1

INTRODUCTION

Turfgrass provides all the major ecosystem services that are provided by other vegetation including; functional, aesthetical, recreational/social and economic services as well as services related to psychological or physical health (Monteiro and Greening, 2017) . About 40% of the total land area used for urban development in the United States is covered by turfgrass (Milesi et al., 2005). There is increasing public demand for high-quality turfgrass on golf courses, parks and urban greenways, residential and institution lawns, and sport fields. These concerns have led to an increase in the intensity of turfgrass management in the United States in recent times (Balogh and Walker, 1992). Superintendents of golf courses and other sport fields use high amounts of conventional inputs (fertilizers, pesticides, fungicides, wetting agents, and plant growth regulators) as means to meet the high-quality turfgrass demand. However, the excessive use of these products has the potential to increase the adverse environmental effects such as runoff losses of applied chemicals into water bodies, harming non-target species and creating more environmental challenges. The environmental concerns associated with high input use as well as the economic implications has put superintendents of golf courses under pressure to reduce the amount of input while at the same time providing high-quality turf. This has led to the proliferation of several turfcare products on the market that are marketed as cheaper and sustainable alternatives to management of golf courses (Karnok and Tucker, 2001a).

Incorporation of wetting agents in the management of golf course and other sports fields has proven to be the most useful tool in addressing the problem of localized dry spots which are

caused by hydrophobic soils on golf greens (Karnok and Tucker, 2001b; Wilkinson and Miller, 1978). Wetting agents are chemical substances that fit into a class of chemicals called surfactant which are used to primarily address the problem of hydrophobic soils on golf greens (Karnok et al., 2004). Plant growth regulators (PGRs) are also applied to golf greens as part of routine maintenance to improve the quality of turfgrass. PGRs are organic compounds, natural or synthetic, that modify or control one or several specific physiological processes within a plant (Lemaux, 1999). In addition to reducing number of mowing, PGRs provide smoother greens and healthier turf (Murphy et al., 2005). Biological products are being used in the routine management of most golf courses as replacement for conventional inputs with prospects of improving plant health. These products are being marketed to improve turfgrass health and overall quality by reducing thatch-mat layer, improving turfgrass nutrient uptake and stress tolerance (Karnok and Tucker, 2001b; McCullough et al., 2007)

Soil microorganisms are of crucial importance to soil health, playing critical roles in nutrient cycling, disease control and degradation of organic matter (Moriarty, 1997; Rousk and Bengtson, 2014). Soil health is defined as the continued capacity of the soil to function as a living system, within ecosystem and land- use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant and animal health (Doran and Safley, 1997).

The use of wetting agents, plant growth regulators, and biological products has aroused interest in soil microbiology in the turf industry over the years. In spite of the wide use of these products in golf course management, there is very little if any information on how these products

impact the soil health. It has become imperative that the impact of these products on soil health is studied. The objectives of this study were;

1. Determine the impact of selected turf care products on soil biological health by measuring soil respiration, urease activity, and phosphatase activity.
2. Determine the impact of selected turf care products on turf quality.

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CHAPTER 2

LITERATURE REVIEW

Golf Course Maintenance

Golf courses are intensively managed ecosystems with high levels of inputs such as fertilizers, pesticides, and other turfcare products. Maintaining quality turf and optimizing irrigation are important goals for superintendents particularly (Oostindie et al., 2008). The playing surface of golf courses tend to receive different treatments; the putting green being the smallest area of the golf course receive the largest proportion of inputs (Schlossberg and Schmidt, 2007). Intensive N fertilization is necessary for maintaining the quality of putting green but present a host of environmental concerns (Liu et al., 2011). Routine application of turf care products in golf courses has become an integral part of management practices in the bid to meet the demand for high-quality turf.

Soil water content plays critical role in the consistency and efficiency of athletic fields (Dickson, 2017). The high volume of water that goes into irrigation of turfgrass systems, even more than the most irrigated crop, corn, in the United States Milesi et al. (2005) make it vital to look at cost-effective ways of managing golf courses. A plethora of microorganisms inhabit the soil and drive processes that make nutrients and water accessible to turfgrass roots. Nitrogen fertilization may stimulate microbial growth and activity as well as the physiology of microbial community thereby impacting organic matter decomposition (Liang and MacKenzie, 1994). Turfgrass systems are under the influence of myriad factors some of which have the potential to

negatively affect the growth and quality of the turf. Heavy rainfall, heat, disease, and thatch accumulation are some of the factors that can affect turf growth and health negatively. Effective management of golf courses present unique challenges to superintendents.

Thatch management is regarded as one of the most important management concerns for most superintendents. Buildup of thatch on golf greens reduce water and nutrient penetration and foster the development of diseases. Thatch is a tightly intermingled layer of living and dead plant tissue that develop between the green vegetation and the soil surface (McCarty et al., 2007). In turfgrass systems, thatch forms as a result of excessive production of organic matter. Mechanical mowing, topdressing and core cultivation are some common practices that are employed to address thatch formation on golf greens (Ledeboer and Skogley, 1967).

A common challenge faced by superintendents is the problem of Localized Dry Spots (LDS) also referred to as dry patch or hot spots (Karnok and Tucker, 2001b). This occurs on golf greens a result of water repellency or diseases, ending with the occurrence of irregular areas on golf greens that show signs of drought (Karnok and Tucker, 2003). Localized dry spot (LDS) is addressed primarily by the application of wetting agents (Karnok and Tucker, 1989). Kostka (2000) reported wetting agents significantly reduced the occurrence of localized dry spot. This study further reported that the application of wetting agents did not have any significant impact on turf quality.

Wetting agents, plant growth regulators, and biological products

Wetting agents (WAs) are compounds that cause a liquid to spread more easily across or penetrate the surface of a solid by reducing the surface tension of the liquid (Zontek and Kostka, 2012). Several wetting agents are available on the market and their use have become a common practice in golf course management regimes. They are primarily applied to make dry soils wet,

enhancing the penetration of water as well as nutrients applied to the surface of golf courses. The chemistry of wetting agents is important in understanding how these products work. Based on their chemistry, they are grouped as anionic, cationic, nonionic, and amphoteric. Anionic and cationic wetting agents ionize in water, liberating their cations and anions in solution which give them their active properties. Nonionic wetting agents do not ionize in water whereas the ability to ionize or not for amphoteric wetting agents depend on the acidity of the solvent (Karnok et al., 2004). Although wetting agents have specific chemical properties associated with each other, they all have a common property of a hydrophilic group attached to a long hydrophobic group (Karnok et al., 2004).

Studies investigating the impact of wetting agents on soil biological health is lacking in literature. However, there are few that have focused on exploring the impacts of wetting agents on turf quality, reducing soil hydrophobicity, and impact on growth of roots. Karnok and Tucker (2001b) reported that wetting agents significantly improved turfgrass color and quality. This study further reported an increase in root length in the 0 to 8 cm depth.

Repellent soils present a huge challenge to superintendents in providing high quality and playable turf. Soil water repellency is a phenomenon of reduced rate of wetting and retention of water in soils caused by the presence of hydrophobic coatings on soil particles. Water repellency affects growth, color, and quality of turfgrass. Hydrophobic coatings causing water repellency are produced by plant root exudates, certain fungal species, surface waxes from plant leaves and decomposing organic matter (Doerr et al., 2005). The severity of water repellency also depends on the proportion of soil particles with hydrophobic coating (Doerr et al., 2005). Wetting agents have proven to be effective in addressing the problem of water repellency. In other areas of agriculture, they have been particularly effective. Madsen et al. (2013) reported that wetting

agents used in irrigated potatoes increased yield up to 20% and also increased tuber quality. Oostindie et al. (2008) also reported improved growth, density, and color of turfgrass on fairways following the application of wetting agents. The study further reported a reduction in water repellency of the soil following the application of wetting agents.

The Florida Department of Agriculture and Consumer Services (FDACS) defines plant growth regulator (PGR) as any substance or mixture of substances that are intended through physiological action, for accelerating or retarding the rate of growth or maturation or for otherwise altering the behavior of ornamental or crop plants or the products thereof, but not including substances intended as plant nutrients, trace elements, nutritional chemicals, plant inoculants, or soil amendments. Most PGRs are applied to inhibit turfgrass growth by interfering with hormonal activity (Johnson, 1989). PGRs are used in modern agriculture, horticulture, and viticulture to regulate many developmental process of plants (Rademacher, 2015). By their modes of action, PGRs are classified into two groups; type I; inhibiting plant growth without causing cell death and type II; inhibiting biosynthesis of gibberellins.

Effective performance of PGRs depend not only on the active ingredients, but also the availability of proper conditions for the product to reach its biochemical target (Rademacher, 2015). In turfgrass systems, the most commonly used PGRs are those that work by inhibiting gibberellin biosynthesis, reducing cell division and foliar elongation (Fagerness and Yelverton, 2001). In spite of the wide use of PGRs in agriculture, their impact on soil biological health has not been widely explored by researchers. Marcum and Jiang (1997) reported that PGRs reduced shoot elongation rate and clipping weight. Another study, Murphy et al. (2005) reported that the quality of St. Augustinegrass was improved after 6 to 10 weeks of treatment with PGRs. Ervin

and Koski (2001) reported that PGRs did not have any significant impact on Kentucky bluegrass root mass.

The high value placed on disease-free golf greens and fairways cause superintendents to frequently apply synthetic fungicides for controlling disease of turfgrass. Excessive application of fungicides raises environmental concerns, fungicide resistance, and hence the increasing concern for alternative ways of controlling turfgrass diseases. Superintendents are turning towards biological control as alternative ways and incorporating the application of biological products (BPs) in their management regimes to ward off pathogens (Noble et al., 2005). BPs applied to turfgrass are a subcategory of a broad group of products generally referred to as biostimulants. Daniels (2013) defined biostimulant as compounds that produce non-nutritional plant growth responses and reduce stress by enhancing tolerance. Biostimulants are available in a variety of formulations and with varying ingredients, including humic substances, hormones, amino acids and microbial inoculants (du Jardin, 2015). Humic substance have shown in previous studies to have positive effect on nutrient uptake. Pascual et al. (1997) showed that humic substances from a number of different parent materials can improve the uptake of total N as well as other nutrients such as P, Mn, Cu, Zn, and Fe. Amino acid containing biostimulants have been shown to increase biomass production (Shehata et al., 2016). Majority of biostimulants used today are complex mixtures of ingredients that are derived from a biological process or extraction of biological material (Yakhin et al., 2017). Billard et al. (2014) reported that biostimulants improve plant productivity through increased assimilation of N, C, and S. BPs such as Plant Helper, Kapre RemeD8-NSL, and Kapre RemeD8-NSP are marketed as useful in mitigating and preventing turfgrass diseases. Elliott et al. (2009) reported that Plant Helper, whose active ingredients is the fungus *Trichoderma atroviride*, inhibited *Phytophthora ramorum*

(sudden oak death) on shrub leaves. The impact of these products on soil biological health is limited in literature.

Soil biological health

The concept of soil health traces its roots to ancient civilizations (Doran and Safley, 1997; Doran and Zeiss, 2000). Healthy soils are key to the maintenance of the integrity of soil ecosystem to withstand stress. Soil health refers to the biological, chemical, and physical features of the soil that are essential to long-term, sustainable agricultural productivity with minimal environmental impact (Arias et al., 2005). Established golf course soils are replete with microorganisms that range in size from microscopic to those seen with the unaided eye. The activities of these microorganisms are very central to the overall functioning of the soil and hence the health of the soil. A healthy soil is resilient to stress, has high biological activity and high levels of internal nutrient cycling (Elliot, 1997). Soil ecosystems support a diversity of microbes (fungi, bacteria and algae), microfauna (protozoa), and mesofauna (arthropods and nematodes) (Neher, 2001).

Free living nematodes play several roles in soils ranging from suppression of diseases to nutrient cycling. Some parasitic nematodes feed on several soil pests, reducing the population of pathogens in soils while other nematodes are plant parasitic. Beare et al. (1997) reported that under field conditions, nematodes contribute about 8% to 19% to nitrogen mineralization in conventional and integrated farming systems respectively. Nematodes play indirect role in nitrogen mineralization by grazing on decomposer microbes, excreting ammonium, and immobilizing nitrogen (Ferris et al., 2004; Ingham et al., 1985).

Protozoans are essential component of the soil ecosystem that consume high proportion of bacterial productivity. Land use changes on agricultural soils have the capacity to modify

protozoan population and affect essential processes that are intricately bound to the health of soil. Protozoans enhance nutrient cycling and energy flow that benefit other soil microorganisms, plants and animals (Foissner, 1999). These microorganisms also enhance the mineralization of ecologically important nutrients such as nitrogen and phosphorus (Ingham et al., 1985).

Bacteria and fungi produce enzyme that help in the breakdown of nutrients in soil and drive many ecosystem functions. They are the most important groups of microbes with regards to nutrient cycling and energy flow in terrestrial ecosystems (Richardson, 2001). Soil microorganisms' response to inputs, and their activities can be used as good indicators of the health of soils. The excessive use of inputs in golf course management could in the long term degrade, sustain, or improve the health of the soil. Soil health is inferred by measuring indicators that are results of soil microbial activity. Some simple and reliable indicators of soil health that have been used in past research focusing on the impacts of inputs on soil microorganisms include microbial abundance, enzyme activity, and rate of soil respiration (Bloem and Breure, 2003).

Soil respiration is the biological oxidation of organic matter to CO₂ by aerobic organisms, particularly microorganisms (Kumpiene et al., 2009). It is a direct measure of how the soil microbial community accept and use inputs to make nutrients available for plant use. Largely, it is measured by capturing the amount of CO₂ emitted. The amount of CO₂ emitted is directly correlated with the activity of soil microbial community. The excessive application of inputs in turfgrass systems challenge soil microbes as their environment is progressively changed by the application of inputs. The response of soil microorganisms to the application of inputs in golf courses is an important indicator of how soil microbial activity is impacted.

Enzyme activity is a useful indicator of the health of soils. Soil enzymes are intimately involved in the process of organic matter decomposition in soil systems by playing key

biochemical functions that are involved in nutrient cycling as well as formation of organic matter (Burns et al., 2013). Soil enzymes play fundamental roles in the maintenance of soil health as vital activators of biochemical processes. The rapid response of soil enzymes to management practices provide an easy, reliable, quick, and cost-effective means of assessing soil health (Dick et al., 1994; Kennedy et al., 1995). Phosphatase and urease are two of the most extensively exploited enzymes used as indicators of soil health. These enzymes play essential roles in P and N cycles respectively. Phosphatases are known for catalyzing hydrolysis of esters and anhydrides of phosphoric acid (Acosta-Martinez et al., 2000). Urease breaks down urea fertilizer added to soil to ammonia and carbon dioxide. Activities of phosphatase and urease provide the means for a meaningful assessment of reaction rates for phosphorus and nitrogen cycling in soil.

The impact of turfcare products on soil biological health has seen a rising interest in recent times. Some past studies have focused on the relationship between the impact of turfcare products on soil and turfgrass growth and quality. Other studies have used enzyme assays, microbial community abundance, and soil respiration as indicators of impact of turfcare products on soil biological health. Karnok and Tucker (2001b) reported that wetting agent treatment significantly improved turfgrass color and quality throughout their entire study. The authors observed no significant difference in color and quality of turfgrass 2 weeks after treatment with wetting agents. However, they reported improved turfgrass color and quality ratings 4, 6, 8, 10, and 12 weeks after treatment. Although color ratings 14, 16, and 18 weeks after treatment were found not be significantly different, turfgrass quality was significantly improved during this period. This study further reported a significant reduction in soil hydrophobicity 3, 5, and 9 weeks after wetting agent treatment.

Mueller and Kussow (2005) investigating the impact of biostimulants on the activity of soil microbial community reported no significant improvement in the rates of enzyme activity. The study further reported a progressive decline in the rates of activity of enzyme activity for the duration of the study. Despite the lack of significant impact on rate of enzyme activity, turfgrass quality was significantly improved.

In spite of efforts made at investigating the impact of management practices on golf systems on turf quality, limited attention has been given to research focusing on the impact of turfcare products on soil health.

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CHAPTER 3

LABORATORY STUDY OF IMPACT OF TURF CARE PRODUCTS ON SOIL
BIOLOGICAL HEALTH

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ABSTRACT

Meeting the demands of high turf quality has been a major preoccupation of superintendents of golf courses and in recent times incorporated wetting agents, plant growth regulators, and biological products in their management regimes. A laboratory incubation study was conducted to investigate the impact of 9 wetting agents (WAs), 4 plant growth regulators (PGRs), and 3 biological products (BPs) on soil biological health. Treatments were applied at 100 x the recommended application rate to simulate the long-term impacts of these products on soil biological health. Sand peat moss mix (90:10) was used for the study and was adjusted to 30% field capacity. Soil samples were analyzed at 5, 10, and 15 days after incubating at 25°C. Soil health was assessed by measuring phosphatase activity, urease activity, and soil respiration as indicators of soil biological health. Among WAs, soil respiration was enhanced by Sixteen 90 and Duplex and suppressed by Vivax and Magnus. Urease activity was enhanced by Vivax, Fleet, Magnus, and Sixteen 90 while Dispatch enhanced phosphatase activity. Among PGRs, Anuew, Cutless, and Proxy enhanced soil respiration, phosphatase, and urease activity respectively. None of BPs impacted the variables of our study.

INTRODUCTION

Turfgrass forms a major component of urban vegetation in the United States, covering an estimated 8 to 10 million hectares (Tashiro, 1987). The benefits of turfgrass cut across the environment, economy, society, and well-being of humans, playing key roles in improving the quality of air, water, and soil (Christians et al., 2011). The ability of turf to absorb atmospheric pollutants help serve as a filter and thus improve the quality of air. Like other agricultural crops, turfgrass has several environmental and aesthetic purposes. Healthy turfgrass systems can serve as buffers for the movement of chemicals from agricultural and urban areas into water bodies. Also, turf plays critical role in protecting soil from water and wind erosion. High concentration of carbon dioxide in the atmosphere has been reported to pose health threats to humans and there are constant efforts to reduce the levels of carbon dioxide in the atmosphere. Qian and Follett (2002) reported that carbon sequestration using grass is a means to achieve this. Turfgrasses are able to absorb carbon dioxide from the atmosphere, reducing the atmospheric levels and fixing carbon in the soil as organic matter (Christians et al., 2011).

The demand for high-quality turfgrass result in high use of fertilizers, pesticides, and water. The environmental concerns associated with high use of inputs in turf maintenance necessitates optimization of resource use efficiency in golf courses. Improving resource use efficiency in golf courses would be cost saving to superintendents of golf courses as well as reduce the detrimental impacts of inputs that are carried by run-offs. Superintendents of golf courses often use wetting agents (WAs), plant growth regulators (PGRs), and biological products (BPs) to enhance the quality of turf as well as improve the resilience of turf to environmental stress. However, the impact of these products on soil biological health has not been extensively investigated.

The focus of golf maintenance has primarily been on improving the quality of turfgrass. Superintendents of golf courses are mostly concerned with improving the quality of the aboveground portion of golf course systems to the neglect of the activities of the soil biota. The activities of the underground life of turf soils are critical to the aboveground portion of golf course systems. Diverse and robust soil microbial activity that drives essential soil processes is vital to the long-term sustainability of turfgrass systems. Soil microbial activities affect the availability or loss of soil N through mineralization or immobilization.

Highly managed turfgrass systems accumulate substantial amount of organic carbon that support soil microbial community. The breakdown of this organic carbon is mediated by a host of soil enzymes (Shi et al., 2006). Soil microbial enzyme activity is mainly of microbial origin that is derived from intracellular, cell-associated or free enzymes. Evaluation of health of turfgrass soils is best approached by assessing parameters that reflect a balance of the physical, biological, and chemical components of soil microbial community (Dick, 2011). Activities of soil enzymes are predominantly used as indices of microbial activity and growth. Soil enzymes play critical roles in the overall process of organic matter decomposition in soil systems by catalyzing several processes essential for the life processes of the soil biota (Sinsabaugh and Soils, 1994). Phosphatase activity, urease activity, and soil respiration rate are some of the most common and reliable methods used in of assessment of soil health.

Maintaining perfect golf greens, reducing the economic costs associated with maintenance, and promoting sustainable management practices has been a major concern for superintendents of golf courses. Established golf course systems like other agricultural systems are under several stress factors. Water repellency and disease outbreak are common challenges that superintendents of golf courses deal with as part of routine management practices. Water

repellency is widespread in established golf course systems and impede the growth and quality of turfgrass (Kostka, 2000). Water repellency occurs due to the coating of soil with hydrophobic organic waxes arriving from leaf surfaces or microbial degradation of plant exudates. Control of water repellency is challenging and costly as several native microorganisms are unable to degrade it once it is established. Application of WAs has proven to be an effective way of controlling water repellency (Kostka, 2000) and hence its widespread application in management regimes of golf course systems.

Golf course systems have incorporated WAs, PGRs, and BPs in their maintenance regimes in order to meet the demand for high quality turf. In established turfgrass systems, WAs have been used as means to improve hydration and enhance irrigation efficiency (Karnok and Tucker, 2001b; Miller et al., 1998). Some studies in the past have reported improved turf quality and reduced soil hydrophobicity following the application of WAs. PGRs work by targeting specific hormones to achieve a desired effect. Generally, in turfgrass systems, the most commonly used plant growth regulators enhance root elongation and suppress shoot growth. Despite the widespread use of these products in turf management, their impact on soil biological health has not received much attention. This study was designed to evaluate the impact of turf care products on soil biological health with time and to simulate the long-term impacts of their repeated applications. The primary objective was to investigate the impact of turf care products on soil microbial activity by assessing rate of soil respiration, phosphatase activity, and urease activity as indicators of soil biological health.

MATERIALS AND METHODS

Sand-Dakota peat moss mixture at a ratio of 90:10 as per recommendations of the United States Golf Association (<http://www.usga.org/Content.aspx?id=25890>) was used for the laboratory incubation study. Moisture content of Sand-Dakota peat moss mixture was determined using equation (1). One-thousand grams of the soil was weighed into Ziploc bag and adjusted to 30% moisture content by following the steps described under “Moisture adjustment” below. The mass of water to add to 1000 g of the soil to bring to 30% moisture content was found to be 135 g. One hundred and thirty-five grams (135 g) of deionized water was weighed into clean cylindrical container and hundred times (100 x) the recommended application rate of product added to it. The cylindrical container was swirled slowly to allow the water and product to mix, the mixture gently poured into a Ziploc bag containing the 1000 g of soil. The Ziploc bag was held at the neck to trap air and shaken vigorously until the soil was uniformly wetted. Four samples of hundred grams (100 g) each of the wetted soil were collected into mason jars and a glass beaker containing 10 milliliters of 0.08-*N* barium hydroxide, Ba(OH)₂, was placed in each mason jar to capture evolved CO₂. Mason jars were incubated in a laboratory incubator set at 25°C for 15 days. An empty mason jar with Ba(OH)₂ trap was used as control to capture background CO₂. Mason jars were removed from incubator at 5, 10, and 15 days after incubation (DAI) and indicators of soil biological health measured. Glass beakers were each replaced and refilled with Ba(OH)₂ after measuring soil biological health indicators at 5 and 10 DAI. WAs were grouped into three sets of three treatments each and the procedure was repeated for all treatments with four replicates.

Table 3.1. Wetting agents (WAs) tested in Laboratory and Greenhouse studies for impact on rate of soil respiration, urease activity, and phosphatase activity.

Product	Manufacturer	Active ingredient	Rate (oz/acre)	% (C, N)
Vivax	Precision Laboratories	Polyethylene polypropylene glycol block polymer	220	65.25, 0.02
Fleet	Harrell's Inc	Polyoxyalkylene polymers Inert ingredients	348	59.55, 0.02
Magnus	Precision Laboratories	Polyethylene polypropylene glycol block polymer	174	63.73, 0.01
Dispatch	Aquatrols	Alkoxylated polyols Glucoethers	8	31.46, 0.03
Revolution	Aquatrols	Modified alkylated polyol	260.9	64.65, 0.05
Sixteen 90	Aquatrols	Propoxylated Polyethylene Glycols	347.8	63.08, 0.02
Cascade	Precision Laboratories	Polyethylene polypropylene glycol block polymer	347.8	63.30, 0.02
Duplex	Precision Laboratories	Alcohol Ethoxylates Alkyl Aryl Sulfonate	20	14.23, 0.45
Pervade	Floratine	Di-sulfosuccinate		20.67, 0.03

Table 3.2. Plant growth regulators (PGRs) and biological products (BPs) tested in Laboratory and Greenhouse studies for their impacts on rate of soil respiration, urease activity, and phosphatase activity.

Product	Product type	Manufacturer	Active ingredient	Rate (oz/acre)	% (C, N)
Trimmit	PGR	Syngenta	Paclobutrazol	200	65.25, 0.02
Proxy	PGR	Bayer	2-chloroethyl phosphonic acid	217	59.55, 0.02
Cutless	PGR	SePRO	5-pyrimidinemethanol	24.6	63.73, 0.01
Anuew	PGR	Nufarm	Prohexadione calcium	37.85	31.46, 0.03
Kapre Reme NSL	BP	Performance Nutrition	Fulvic acid, Kalpene Greens, NutriSmart WSP	780	0.74, 0.07
Kapre Reme NSP	BP	Performance Nutrition	Fulvic acid, NutriSmart WSP	43	30.05, 2.43
Plant Helper	BP	Precision Laboratories	<i>Trichoderma</i> <i>atroviride</i>	80	32.81, 0.72

Moisture content at field capacity

To determine the moisture content at field capacity, Sand-Dakota peat moss was collected in three separate funnels fitted with filter paper and wetted. The set up was allowed to stand overnight to allow water to drain through the filter paper. 10 g of soil from each funnel was collected into aluminum plates and dried in an oven set 100°C for 24 hours. At the end of the 24-hour period, soil samples were allowed to cool in a desiccator for 2 hours and oven dry-weight of the soil was measured. Water holding capacity of the soil was calculated using the equation (1) below;

$$\text{Water holding capacity (\%)} = \frac{\text{wet soil weight} - \text{OD soil weight}}{\text{weight of oven-dried soil}} \times 100\% \quad (1)$$

Moisture adjustment

For the incubation study, the soil moisture content was adjusted to 30% field capacity. Moisture content of soil at field capacity was found to be 12.69%

Data Collection

Soil respiration rate, phosphatase activity, and urease activity were the soil biological health indicators measured at 5, 10, and 15 DAI. Jars were brought out of the incubator and analyzed for treatment impact on soil biological health.

Soil respiration

Mason jars were brought out of incubator and all four replicates of each treatment were analyzed for rates of soil respiration (mg CO₂ evolved g⁻¹) as indicator of the overall effect of each treatment on soil microbial activity (Kennedy et al., 1995). Using phenolphthalein as indicator, 0.08-N HCl was used to titrate the Ba(OH)₂ trap in each mason jar. The volume of HCl used to titrate each Ba(OH)₂ trap until endpoint was recorded. CO₂ from control jar was

subtracted from jars with soil and equation (7) was used to estimate rate of soil respiration, where y was the volume of HCl used in titrating each trap until endpoint.

$$\text{mg CO}_2 \text{ g}^{-1} = \left[\frac{(0.08 \text{ N Ba(OH)}_2 \times y \text{ mL Ba (OH)}_2) -}{(0.08 \text{ HCl} \times y \text{ mL HCl})} \right] \times \frac{22 \text{ mg CO}_2}{10 \text{ g} \times \text{soil weight (g)}} \quad (2)$$

Urease activity

Soil from each mason jar was collected and analyzed with 2% boric-acid indicator to estimate the rate of urease activity ($\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{ h}^{-1}$). The rate of urease activity was analyzed as an indicator of soil N cycling, Lloyd et al. (1973) as per (Mobley et al., 1989). For each replicate of each treatment, two biplate petri dishes were prepared by adding 1g of soil in one compartment. Three milliliters of 0.5-M Tris-maleate buffer solution (pH 7.0) with 1% sodium azide was added to the soil in each plate. Three milliliters of 2% boric acid indicator was pipetted into the other compartment of each biplate petri dish. One milliliter of 6-M urea was added to the soil-buffer solution in one replicate of each treatment to initiate the reaction. The soil-buffer compartment of the second biplate petri dish was treated with one milliliter of distilled water to account for the release of ammonia (NH_3) from background ammonium (NH_4^+). Petri dishes were allowed to incubate at room temperature for one hour. After incubation, one-half milliliters of 10 mM AgSO_4 solution and 3-M K_2CO_3 were added to both plates of each replicate of each treatment to terminate urease activity and release evolved NH_3 into the boric acid trap. Plates were secured in Ziploc bags and allowed to incubate at room temperature for 24 hours. After the 24-hour incubation, boric acid solutions were titrated with 0.02-N HCl. The rate of urease activity was calculated for each replicate by applying equation (4) and subtracting the value of the control from the treatment.

$$\mu\text{mol NH}_3 \text{ evolved } \text{g}^{-1} \text{ h}^{-1} = \frac{\text{mL HCl}}{\text{soil weight (g)}} \times \frac{0.02 \text{ mol}}{\text{L}} \times \frac{1 \text{ L}}{1000 \text{ mL}} \times \frac{10^6 \mu\text{mol}}{\text{mol}} \quad (3)$$

Phosphatase activity

Soil samples were analyzed for rate of phosphatase activity as indicator of soil P cycling as per (Tabatabai, 1994). Two 16-milliliter glass scintillation vials were obtained for each replicate of each treatment and wrapped in aluminum foil to reduce light exposure. One gram of soil and 4 milliliters of Tris-maleate buffer (pH 7.0) were added to each vial. One milliliter of 100-mM para-nitrophenyl phosphate (pNPP) was added to one of the two vials and the other used as control. All treatment and control vials were shaken on a rotary shaker at 175 rpm for 30 minutes after which 1 milliliter of pNPP was added to the control vials. One milliliter of 0.5- CaCl_2 and 4 milliliters of 0.5-M NaOH were added to all vials to terminate the phosphatase activity in each vial. Contents of each vial were then transferred to 16- milliliter polystyrene centrifuge tubes and centrifuged at 10,000 rpm for 10 minutes at 4°C. The absorbance of the supernatant from each vial was colorimetrically analyzed using a spectrophotometer set at 400 nm. Samples were diluted 1:25 when the absorbance was too high to detect. Standard solutions ranging from 0 to 7.5 μM p-nitrophenol in Tris-maleate buffer were used to derive standard curves. Linear equations derived from standard curves ($R^2 > 0.9905$) were used to calculate pNPP concentrations (which is equivalent to the released phosphate) in each vial. The difference between phosphate concentrations in the treated and control vials were used in equation (4) to determine the phosphatase activity ($\mu\text{mol P evolved g}^{-1} \text{ h}^{-1}$) in each soil sample.

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

Statistical analysis

All statistical analyses were performed using JMP Pro 13. Each treatment was analyzed in replicates of four and values averaged. Treatment-time interaction effects were examined by one-way analysis of variance (ANOVA). Differences between treatment means and controls were evaluated with Dunnet's test with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Soil respiration

Sixteen 90 has a comparatively high carbon content (Table 3.1), suggesting that carbon in this product might have become available to soil microbes over time. Dispatch and Revolution did not have any significant impact on rate of soil respiration. Although Dispatch has high carbon content (Table 3.1), the carbon in this product might have been bound in ways that are not easily degradable by soil microbes and hence their lack of impact on rate of soil respiration.

The BPs had comparatively lower carbon contents (Table 3.2) which is a plausible reason for their lack of significant impact on rate of soil respiration.

The enhanced rate of soil respiration observed for Anuew at 15 DAI suggest Anuew might have acted as carbon source for soil microbes. Although Anuew has a relatively lower carbon content among PGRs (Table 3.2), the carbon in Anuew might have been bound in ways that are easily degradable, serving as readily available carbon sources for soil microbes and hence the enhanced rate of soil respiration. A plausible explanation for the lack of impact on soil respiration by Anuew in the early days of the incubation study (5 and 10 DAI) is that microbial response to carbon sources in Anuew is not immediate. Impact of WAs on rate of soil respiration fluctuated during the duration of the study. Sixteen 90, Revolution, Duplex, and Pervade showed no significant impact on soil respiration at 5 DAI (Table 3.3). This indicates that microbial

community did not respond to the application of these products in the initial stage of the study. However, Dispatch, Fleet, Magnus, and Vivax suppressed rate of soil respiration at 5 DAI (Table 3.3). This implies that the application of these products resulted in the decline of soil microbial activity. A plausible reason for this observation is that active ingredients of these products might have some antimicrobial properties and hence the suppressed rate of soil respiration.

Duplex and Sixteen 90 showed improved rate of soil respiration although when compared with the control, rates were not significantly different from the control (Table 3.3) at 10 DAI. This suggests that microbial community responded slowly to the application of these treatments. A plausible explanation is that these treatments had the capacity to enhance microbial activity over time. The pattern shown by Duplex and Sixteen 90 could be as a result of these treatments containing carbon sources that may be bound in ways that are not readily liberated into the soil as organic sources for microbial community to act up. Magnus, Fleet, Revolution, and Vivax suppressed soil respiration at 10 DAI (Table 3.3), implying that the application of these treatments inhibited microbial activity. This inhibitory impact observed for Vivax and Fleet in this study agrees with the findings of Song et al. (2019) which reported that alkyl block polymers (active ingredients in Vivax and Magnus) showed inhibition of microbial populations, restricting availability of carbon for soil microbial microorganism after repeated applications. At 15 DAI, Pervade, Duplex, and Sixteen 90 significantly improved soil respiration when compared with the Control (Table 3.3). This implies that Duplex, Pervade, and Sixteen 90 served as carbon sources for soil microbes.

Rate of soil respiration for PGRs ranged from $0.52 \text{ mg CO}_2 \text{ g}^{-1}$ to $1.55 \text{ mg CO}_2 \text{ g}^{-1}$ at 5 DAI. When rates were compared with the control, Anuew did not have significant impact on rate of soil respiration whereas Proxy, Trimmit, and Cutless significantly impacted rate of soil

respiration at 5 DAI (Table 3.4). Rates for Trimmit, Cutless, and Proxy were significantly lower than the Control indicating a suppressive impact on rate of soil respiration by these treatments. The active ingredients in these products might have had antimicrobial effects and hence the reduced rate of soil respiration. The reduced rate of soil respiration by Trimmit observed in this study agrees with the findings of Silva et al. (2003) which reported negative impact of paclobutrazol (active ingredient in Trimmit) on soil microbial community. Silva et al. (2003) further reported that the application of paclobutrazol reduced dehydrogenase activity, which directly correlates to reduced rate of breakdown of organic matter in the soil as does rate of soil respiration. Anuew significantly impacted rate of soil respiration while Cutless, Proxy, and Trimmit showed no significant impact (Table 3.4) at 15 DAI. Rate of soil respiration recorded for Anuew averaged $0.946 \text{ mg CO}_2 \text{ g}^{-1}$ at 15 DAI and was significantly higher than the Control ($0.33 \text{ mg CO}_2 \text{ g}^{-1}$). Cutless and Trimmit suppressed rate of soil respiration while Anuew and Proxy did not have any significant impact on rate of soil respiration at 10 DAI (Table 3.4).

There was no significant interaction between treatments and time for impact of BPs on rate of soil respiration (Table 3.5). When mean rates of BPs were compared with the Control, none of the treatments significantly impacted rate of soil respiration (Figure 3.1).

Table 3.3. Impact of wetting agents applied to a mixture of sand/peat moss (90:10) at 100 x the recommended rate on rate of soil respiration in a 15-day Laboratory study. CO₂ emitted at each sampling time is compared against their respective Control.

Treatment	5 DAI (mg CO ₂ g ⁻¹)	10 DAI (mg CO ₂ g ⁻¹)	15 DAI (mg CO ₂ g ⁻¹)
Vivax	0.60b	0.42b	0.10b
Dispatch	0.59b	1.07a	0.89a
Pervade	1.53a	0.94a	0.98a
Control	1.55a	0.97a	0.85a
Fleet	0.52b	0.41b	0.85a
Magnus	0.52b	0.41b	0.083b
Cascade	0.86a	0.97a	0.83a
Control	0.88a	0.97a	0.85a
Sixteen 90	1.43a	0.97a	0.68a
Revolution	1.39a	0.91a	0.59b
Duplex	1.43	0.91a	0.86a
Control	1.40a	0.89a	0.48b

¹DAI = Days after incubation. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's test.

Table 3.4. Impact of plant growth regulators applied to a mixture of sand/peat moss (90:10) at 100 x the recommended rate on rate of soil respiration in a 15-day laboratory study. CO₂ emitted at each sampling time is compared against their respective Control.

Treatment	5 DAI	10 DAI	15 DAI
	(mg CO ₂ g ⁻¹)	(mg CO ₂ g ⁻¹)	(mg CO ₂ g ⁻¹)
Trimmit	0.66b	0.49b	0.30b
Cutless	0.52b	0.51b	0.28b
Proxy	1.08b	0.95a	0.36b
Anuew	1.55a	0.96a	0.95a
Control	1.58a	1.04a	0.33b

¹DAI = Days after incubation. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's test

Table 3.5. One-way ANOVA of impact of biological products on rate of soil respiration over 5, 10, and 15 days after incubation.

Source	DF	F Ratio	Prob > F
Treatment	3	2.9539	0.0674
Time	2	184.299	<.0001
Treatment*Time	6	06278	0.5406

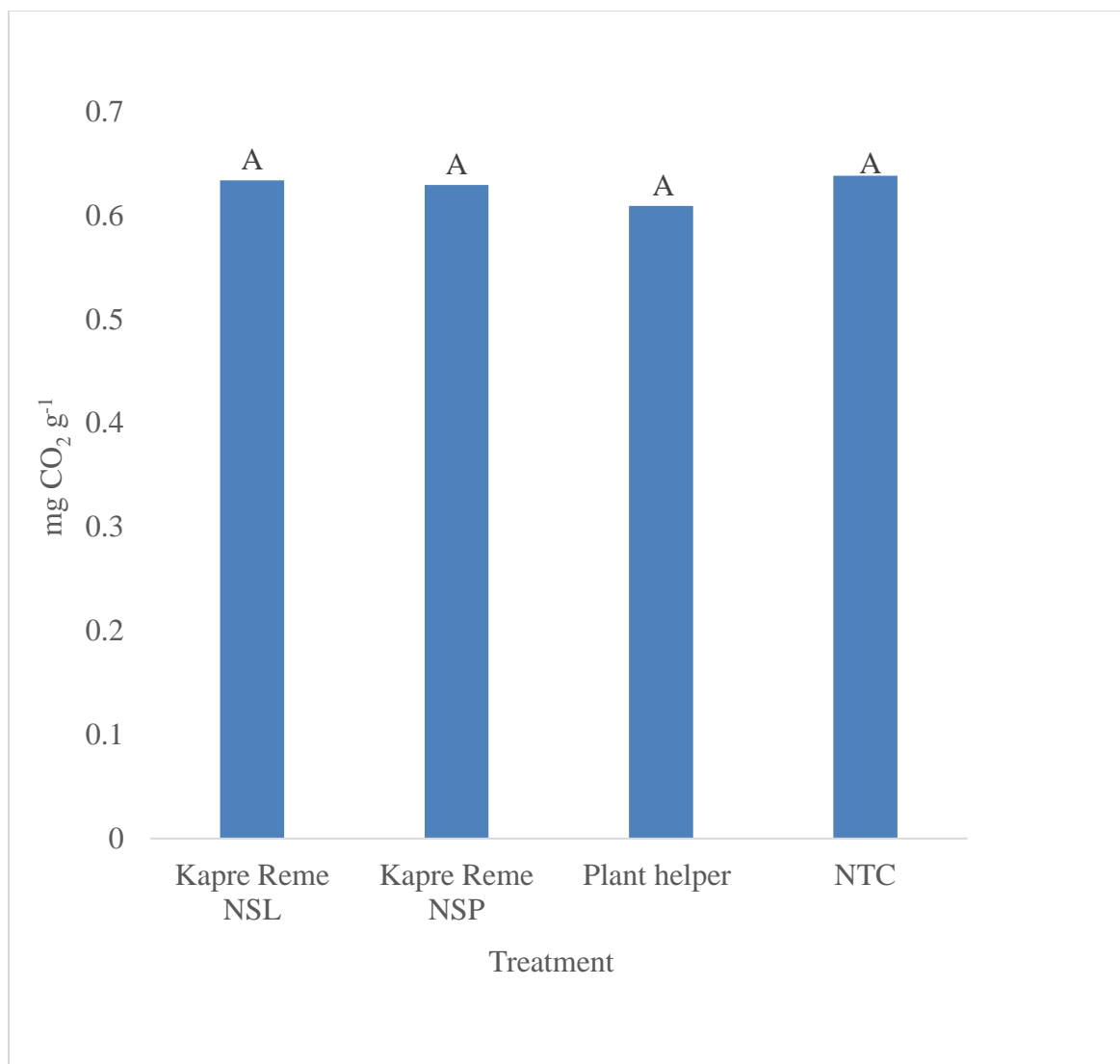


Figure 3.1. Mean impact of biological products applied to a mixture of sand/peat moss (90:10) at 100 x the recommended rate on rate of soil respiration in a 15-day Laboratory study.

Urease activity

WAs impacted urease activity to different degrees ranging from 1 $\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{h}^{-1}$ to 17 $\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{h}^{-1}$ for the 15-day incubation period. WAs had unchanged, enhanced, or suppressed impact on urease activity. Nitrogen content of these products are very small (Table 3.1) and thus might have not acted as significant sources for of nitrogen for soil microbes. Vivax, Fleet, and Magnus significantly improved urease activity at 5 DAI (Table 3.6.) However, these WAs might have enhanced the infiltration of water into the soil thereby increasing the moisture content of the soil and hence urease activity. This agrees with the findings of Sahrawat and Soil (1984) which concluded that increased availability of water enhances urease activity. When compared with the Control, none of Revolution, Pervade, Sixteen 90, Cascade, and Duplex had significant impact on urease activity while Dispatch inhibited urease activity at 5 DAI (Table 3.6).

Pervade, Dispatch, Duplex, Cascade, sixteen 90 had no significant impact on urease activity 10 DAI (Table 3.6). This implies that the application of these products did not enhance or inhibit urease activity. Revolution, Vivax, Fleet, and Magnus significantly impacted urease activity 10 DAI. This was consistent with the observation 5 DAI except for Revolution. The significant impact on urease activity for Revolution at 10 DAI indicates that the impact of Revolution on ureolytic microbial activity following application is not immediate. The increase in urease activity observed for Revolution 10 DAI is presumably due to the fact that over time, Revolution caused significant wetting of the soil enhancing infiltration and subsequent increase in urease activity. Vivax and Fleet significantly enhanced urease activity at 10 DAI.

At 15 DAI, Vivax, Fleet, and Magnus maintained the pattern recorded 5 and 10 DAI, significantly enhancing urease activity (Table 3.6). Mean rates of urease activity for Vivax,

Fleet, and Magnus were highest 15 DAI. Revolution, Sixteen 90, and Duplex significantly impacted urease activity 15 DAI (Table 3.6). The significant impact of Vivax, Fleet, and Magnus, sixteen 90, Revolution, and Duplex is possibly due to the lysis of microbial cells and subsequent release of urease to degrade urea and its derivatives (Ladd and Jackson, 1982; Lai et al., 1992). Urease activity of BPs ranged from $10 \mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$ to $14 \mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$. Activity of urease did not significantly vary over time. The highest mean rate was recorded for Plant Helper and least for Kapre Reme NSP. However, when rates were compared with the control, none were significantly different (Figure 3.2). This indicates that BPs did not enhance urease activity. A plausible reason is that these products could cause an increase in microbial activity that would result in increased production of urease.

Among the PGRs, Anuew and Proxy showed significant impacts on urease activity 5 DAI (Table 3.7). Urease activity for Anuew was significantly lower than the rate recorded for the control. This indicates suppressive impact of Anuew on urease activity. To the contrary, Proxy significantly enhanced urease activity. Urease activity recorded for Proxy 5 DAI was $14.25 \mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$. No significant impact on urease activity was recorded for Cutless and Trimmit 5 DAI. Urease activity for Anuew increased at 10 DAI (Table 3.7) but remained non-significant when compared with the Control. A plausible explanation for the sharp increase in urease activity for Anuew is that ureolytic enzymes that may have been suppressed in the early stages following the application of Anuew became active over time. Proxy continued to significantly impact urease activity at 10 DAI while Cutless and Trimmit did not have any significant impact on urease activity.

Urease activity for Proxy, Cutless, and Trimmit all declined. When compared with the control, rate for Proxy was significantly higher at 15 DAI (Table 3.7). The progressive increase

in urease activity for Vivax, Fleet, and Magnus might be due to the capacity of these products to cause an increase in microbial activity over time due to their relatively high carbon sources. The carbon sources in these products might have served as substrates for soil microbes and slowly degraded by soil over time. Duplex, Revolution, and Sixteen 90 significantly enhanced urease activity at 15 DAI as against their suppressive impacts at 5 and 10 DAI. This is presumably because catalysis of the components of Duplex, Revolution, and Sixteen 90 might have released metabolites that were antagonistic to urease during the early stages of the incubation. Active ingredients of these products might have had antimicrobial properties that suppressed enzyme activity in the early stages of the study. The low rate of urease activity at 5 and 10 DAI agrees with the findings of Singh and Nye (1984) which reported that during incubation, free urease is attacked by protease which potentially reduce the amount and activity of urease in soil.

Table 3. 6. Impact of wetting agents applied to a mixture of sand/peat moss (90:10) at 100 x the recommended rate on urease activity ($\mu\text{mol NH}_3 \text{ emitted g}^{-1} \text{ h}^{-1}$) in a 15-day Laboratory study.

Rates at each sampling time is compared against their respective Control.

Treatment	5 DAI	10 DAI	15 DAI
Vivax	6.8a	8.5b	13.5a
Dispatch	2.5b	12.0a	10.5b
Pervade	4.0a	12.0a	9.5b
Control	4.5a	10.3a	10.0b
Fleet	14.8a	12.0a	9.5a
Magnus	14.5a	23.5a	3.3a
Cascade	6.3b	9.3b	4.8a
Control	6.0b	9.8b	1.3b
Sixteen 90	8.8a	8.0b	12.0a
Revolution	6.8a	16.0a	14.0a
Duplex	6.5a	9.0b	14.0a
Control	6.5a	6.5b	6.8b

¹DAI = Days after incubation. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's test.

Table 3.7. Impact of plant growth regulators applied to a mixture of sand/peat moss (90:10) at 100 x the recommended rate on urease activity ($\mu\text{mol NH}_3$ emitted $\text{g}^{-1} \text{h}^{-1}$) in a 15-day Laboratory study. Rates at each sampling time is compared against their respective Control.

Treatment	5 DAI	10 DAI	15 DAI
Trimmit	10.0b	8.0b	8.0b
Cutless	9.5b	8.0b	8.0b
Proxy	14.0a	13.0a	13.0a
Anuew	5.0c	5.0c	9.0b
Control	10.0b	10.0b	10.0b

¹DAI = Days after incubation. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's test.

Table 3.8. One-way ANOVA of impact of biological products on urease activity over 5, 10, and 15-day incubation period.

Source	DF	F Ratio	Prob > F
Treatment	3	0.6105	0.63138
Time	2	7.7888	0.0094
Treatment*Time	6	1.2547	0.3089

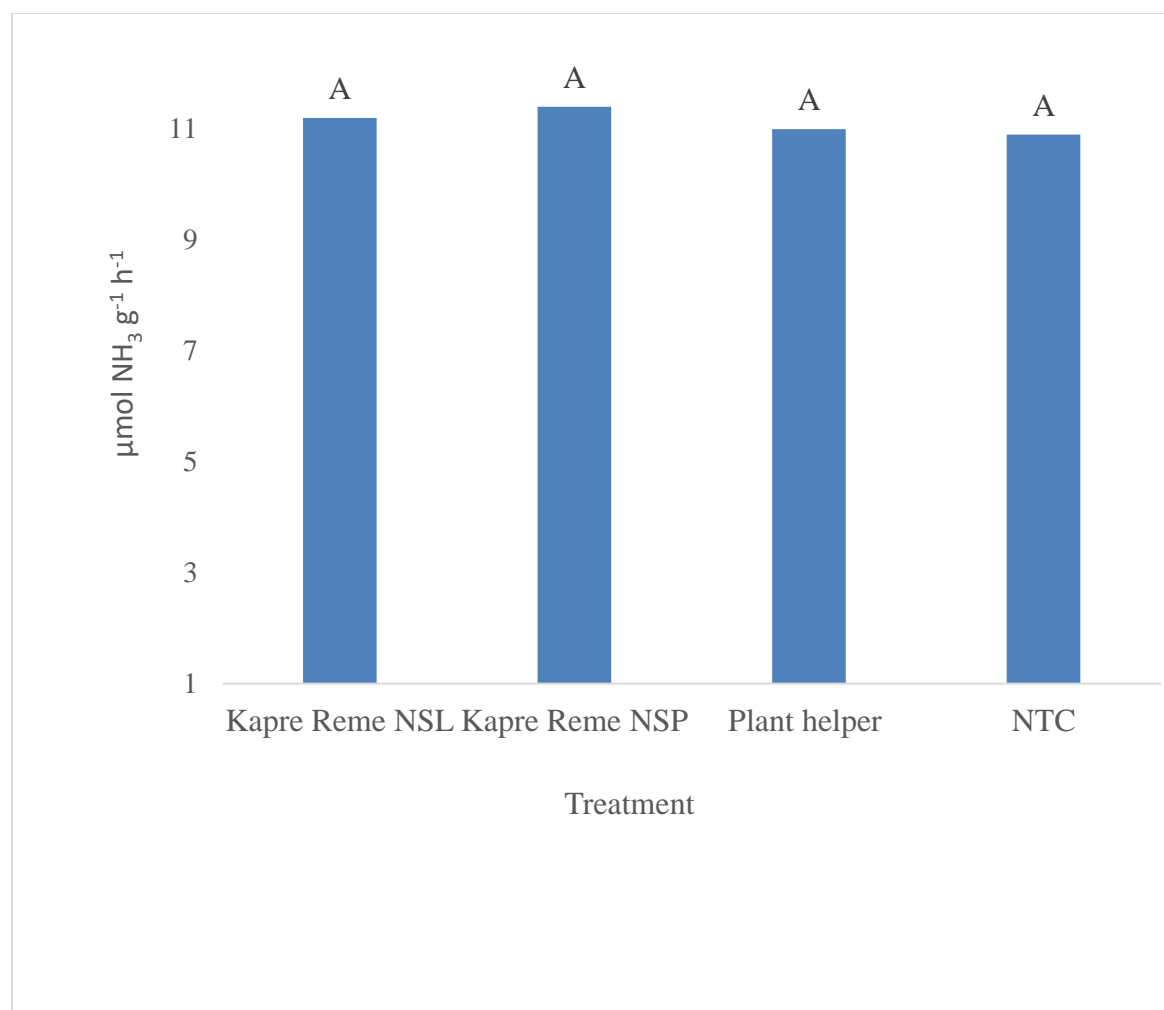


Figure 3.2. Mean impact of biological applied to a mixture of sand/peat moss (90:10) at 100 x the recommended rate on CO₂ emitted in a 15-day Laboratory study.

Phosphatase activity

Mean phosphatase activity for wetting agents ranged from 0.78 $\mu\text{mol Pi evolved g}^{-1} \text{ h}^{-1}$ to 46.2 $\mu\text{mol Pi evolved g}^{-1} \text{ h}^{-1}$ for the 15- day incubation study. Among the WAs, phosphatase activity was significantly enhanced by Dispatch and suppressed by Cascade at 5 DAI when treatments were compared with the Control (Table 3.9). Dispatch significantly enhanced phosphatase activity at 10 DAI.

At 15 DAI, there was no significant change in the pattern observed at 5 and 10 DAI except for Dispatch which showed an increase in rate of phosphatase activity and was significantly higher when compared with the Control whiles Magnus, Cascade, and Revolution suppressed phosphatase activity (Table 3.9). For WAs that did not enhance phosphatase activity, a plausible reason is that these WAs could not cause the release of bound phosphatase during the duration of our study or could not increase microbial activity that would lead to increased production of phosphatase or both. The positive impact of Dispatch on phosphatase activity might be due to Dispatch causing a release of bound phosphatase enzyme and stimulating its activity or the availability of phosphorus in this product or both. An alternative explanation for the impact of Dispatch on phosphatase activity is that this product might have caused an increase in bacterial activity following the application of Dispatch (Nannipieri et al., 2011).

Phosphatase activity for PGRs ranged from 0.37 $\mu\text{mol Pi evolved g}^{-1} \text{ h}^{-1}$ to 1.69 $\mu\text{mol Pi evolved g}^{-1} \text{ h}^{-1}$. Anuew suppressed phosphatase activity at 5 DAI whiles Trimmit, Cutless, and Proxy had no significant impact on phosphatase activity (Table 3.10). Anuew continued to suppress phosphatase activity through to 15 DAI. Cutless enhanced phosphatase activity whiles the other PGRs had no significant impact (Table 3.10). This suggests that Cutless might have

acted as additional source of phosphorus for soil microbes or increased microbial activity leading to increased production of phosphatase or both.

Table 3.9. Impact of wetting agents applied to a mixture of sand/peat moss (90:10) at 100 x the recommended rate on phosphatase activity in a 15-day Laboratory study. Rates at each sampling time are compared against their respective Control.

Treatment	5 DAI	10 DAI	15 DAI
	($\mu\text{mol Pi g}^{-1} \text{ h}^{-1}$)	($\mu\text{mol Pi g}^{-1} \text{ h}^{-1}$)	($\mu\text{mol Pi g}^{-1} \text{ h}^{-1}$)
Vivax	5.6b	0.78b	1.7b
Dispatch	32.1a	25.9a	45.8a
Pervade	16.0 b	11.5b	13.2b
Control	9.5b	7.9b	13.1b
Fleet	32.3a	28.3a	32.3a
Magnus	39.7a	25.9a	22.6b
Cascade	19.2b	22.5a	28.3b
Control	39.7a	26.4a	32.9a
Sixteen 90	46.2a	13.8a	32.3a
Revolution	31.0a	13.6a	22.6b
Duplex	31.7a	13.0a	28.3a

Control	34.9a	12.9a	32.9a
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¹DAI = Days after incubation. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's test

Table 3.10. Impact of plant growth regulators applied to a mixture of sand/peat moss (90:10) at 100 x the recommended rate on phosphatase activity in a 15-day Laboratory study. Rates at each sampling time are compared against their respective Control.

Treatment	5 DAI	10 DAI	15 DAI
	($\mu\text{mol Pi g}^{-1} \text{ h}^{-1}$)	($\mu\text{mol Pi g}^{-1} \text{ h}^{-1}$)	($\mu\text{mol Pi g}^{-1} \text{ h}^{-1}$)
Trimmit	1.69a	1.69a	1.21b
Cutless	1.62a	1.62a	1.53a
Proxy	1.49a	1.49a	1.14b
Anuew	0.37b	0.37b	1.23b
Control	1.50a	1.50a	1.19b

CONCLUSIONS

BPs did not significantly, positively or negatively impact the variables used in assessing soil biological health for this study (soil respiration, phosphate activity, and urease activity). Vivax, Fleet, Magnus, and Sixteen 90 enhanced urease activity. Rate of soil respiration was enhanced by Sixteen 90 and Duplex whereas Vivax and Magnus suppressed it. The impact of treatments on soil respiration fluctuated as a declining pattern in rate of soil respiration was observed for some treatments and the contrary for others. Among PGRs, Anuew enhanced rate of soil respiration with Cutless enhancing phosphatase activity. Proxy was the only PGR that enhanced urease activity. The results of this study highlight the fate of turfcare products in soil as related to improving, sustaining or declining the overall soil biological health. The results of this study add to understanding the dynamics of the impact of turfcare products. In practical sense, this study provides information for golf course superintendents and soil scientist alike with regards to sustaining the health of turf soil systems.

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CHAPTER 4

GREENHOUSE STUDY OF IMPACT OF TURF CARE PRODUCTS ON TURF QUALITY AND SOIL BIOLOGICAL HEALTH

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ABSTRACT

A fourteen-week study was conducted in a greenhouse to study the impact of turfcare products on turf quality and soil biological health. 9 wetting agents (WAs), 4 plant growth regulators (PGRs), and 3 BPs were sprayed on potted bentgrass for 7 and 14 weeks using the Generation 3 Research Track Sprayer at treatments recommended rates. Images of grass were taken with the light tube at 7 and 14 weeks after treatment and analyzed with FieldAnalyzer software. Soil samples were collected at the 5 cm depth after 7 and 14 weeks and analyzed for phosphatase activity, urease activity, and rate of soil respiration as indicators of soil biological health. To investigate if the application of these treatments enhanced the disease suppressive nature of the soil, the grass was inoculated with dollar spot pathogen and the progression of the disease studied for 40 days. Among WAs, Vivax, Fleet, Magnus, and Dispatch enhanced rate of soil respiration while Revolution and Duplex enhancing urease activity. Among PGRs, Cutless, Trimmit, and Anuew suppressed rate of soil respiration while phosphatase activity was not impacted by any treatment. Pervade, Revolution, Sixteen 90, Cascade, Duplex and Kapre Reme NSL, a BP improved turf quality. No treatment improved the disease suppressive nature of the soil.

INTRODUCTION

Putting greens have been reported to deteriorate in the absence of the positive influence that an active microbial community can have on soil health (Hodges, 1990). Some of the products used in the maintenance of turfgrass are marketed as having the capacity to increase microbial density and activity (Mueller and Kussow, 2005). The extensive use of these products in golf management practices have the capacity to alter soil microbial communities since turfgrass ecosystems support abundant populations of different microorganisms. The decomposition of turfgrass roots support diverse and robust soil microflora and microfauna. Turfgrass roots and soil microbial community form relationships that are largely beneficial to the growth of turfgrass (Martens and Frankenberger, 1993).

Soil health is of importance to turfgrass management as the quality of turfgrass depends on the health of soil. Soil enzymes are critical in maintaining soil health by their active roles in decomposition of organic matter and nutrient cycling. Their response to management practices are used as reliable indicators of soil health (Dick et al., 1994) . High rates of enzyme activity correlate with high microbial activity and healthy soils. Assessing specific enzyme response to application of inputs to turfgrass and turfgrass soils could give vital information on the health of turfgrass soils.

Disease outbreaks and their management are major concerns for superintendents of golf courses. Diseases reduce the aesthetic and playing quality of turfgrass and hence the considerable efforts at reducing their occurrence on golf greens. Dollar spot (*Clarireedia* spp. formerly *Sclerotinia homoeocarpa*) is one of the diseases of economic importance in the turfgrass industry (Walsh et al., 1999). Dollar spot can cause considerable damage to highly maintained golf course

putting green as well as closely mown fairways (Workman, 2012). The disease affects many turfgrass species in various parts of the world (Fidanza et al., 2006). Soils have the natural ability to suppress pathogens to a certain extent and the success of a pathogen is influenced by the microbial community of the soil in which the infection takes place. Enhanced disease suppressiveness can be achieved by the application of organic amendments by stimulating soil microbial activity (Hoitink and Boehm, 1999). Soil microbial community is essential in preventing disease outbreaks in turfgrass systems. Some past studies have focused on investigating the impact of turfcare products on turf quality. Karnok and Tucker (2001b) reported that wetting agents did not improve color and quality of turfgrass 2 weeks after treatment. However, a significant increase in color and quality was observed after 4, 6, 8, 10, and 12 weeks after incubation. Despite their findings, the authors concluded could not conclude with certainty that studies with other wetting agents would have similar results. (Mueller and Kussow, 2005) reported that biostimulant application increased enzyme activity temporarily.

In spite of the excessive use of wetting agents, plant growth regulators and biological products in golf courses, their impact on soil biological health remains not much studied. Studies on whether these products have any impact on disease suppressive nature of soils was not found in literature. This study was designed to study whether the application of wetting agents, plant growth regulators, or biological products could enhance the disease suppressive nature of soil. The primary objective of this study was to determine the relationship between turf quality and soil biological health.

MATERIALS AND METHODS

Experimental set-up

A fourteen-week study was conducted at a greenhouse of University of Georgia Griffin Campus from January 14, 2019–October 15, 2019. Cylindrical pots of diameter 6.5 centimeters were filled to the brim with and peat and peat moss mix (ratio of 90:10) as per the recommendation of the United States Golf Association (USGA). Pots were seeded with bentgrass (*Agrostis stolonifera*) seeds and watered twice daily. Grass continued to receive only water from January 2019 to March 2019 until pots were fully covered by grass. Weekly cutting of grass was started from April 2019. Prior to treatment application, all pots were fertilized twice with Glo Pro at rate of 0.1 pounds per 1000 square feet. Pots were randomly categorized into two sets. The first set of pots received treatment application of 4 wetting agents (WAs) and 4 plant growth regulators (PGRs) and the second, 5 WAs and 3 biological products (BPs). Each treatment was mixed in 30 milliliters of deionized water and sprayed on four replicates of each treatment using Generation 3 Research Track Sprayer (Devries Manufacturing, Hollande, MN) with pressure set between 40 and 45 psi.

Weekly application of treatments at recommended application rates was started in May 2019 and lasted 14 weeks. After each spraying, pots were transported to cool greenhouse with temperature range of 15°C to 23°C. Pots were kept in cool greenhouse and grass cut once every week. Daily watering of grass continued except for days when treatments were sprayed. 7 and 14 weeks after treatment (WAT), images of grass were taken with light tube fitted with a camera and soil samples collected for laboratory analyses. Grass was inoculated with Dollar spot pathogen of C3 grasses (*Clarireedia jacksonii*) isolated from bentgrass on University of Georgia Griffin Campus 48 hours after sampling for lab analyses. Five centers on each pot were

inoculated with the Dollar spot inoculum and inoculated grasses were kept in the warm greenhouse in a closed shed with water at the base. The temperature in the shed was 28°C with high and low humidity 99% and 92% respectively. Pots were kept in the shed for 24 hours for Dollar spot to grow and progression of disease was studied by visually assessing disease severity using a modified version of the Barratt and Horsfall (1945) on a scale of 1 to 11, 1 being the least infected and 11 the worst at 10, 20, 30, and 40 days after inoculation.

Sample Collection and Analysis

Soil samples were collected from the rhizosphere (soil-root contact region of active microbial and enzyme activity) of the grass to a depth of 5 cm at 7 and 14 WAT. Auger was used to randomly sample soil and holes left behind were filled with sand after each sampling. Soil samples were stored in Ziploc bags and transported on ice to the lab. Samples were processed through a 2 mm sieve to remove plant debris. Sieved soil samples were then analyzed for rate of soil respiration, phosphatase, and urease activities. Images of grass were taken at 7 and 14 WAT and images analyzed using the FieldAnalyzer software. 48 hours after samples were taken for lab analyses, grass was inoculated with Dollar spot pathogen. Five centers on each pot were inoculated with the Dollar spot inoculum and inoculated grasses were kept in the warm greenhouse in a closed shed with water at the base. The temperature in the shed was 28°C with high and low humidity 99% and 92% respectively. Pots were kept in the shed for 24 hours for dollar spot to grow. After 24 hours of being in the shed, the pots were transported to a cool greenhouse. Pots were watered daily to reduce water stress and, progression of disease studied over a forty-day period. Disease progression was studied by counting infection centers and assigning degree of disease severity using the modified Barratt and Horsfall (1945) on a scale of 1 to 11 with 1 = least severe and 11 = most severe at 10, 20, 30, and 40 days after inoculation.

Soil respiration

10 g of soil from each replicate was weighed into separate empty mason jars. Four empty mason jars served as controls to capture background CO₂. Glass beakers were filled with 10 milliliters of 0.08 -N Ba(OH)₂ and placed in each mason jar to capture evolved CO₂. Jars were sealed tightly and grouped according to treatments in Ziploc bags. Jars were allowed to incubate in the dark at room temperature for 24 hours. Using phenolphthalein as indicator, 0.08-N HCl was used to titrate the Ba(OH)₂ traps. CO₂ from the Control jars were subtracted from jars with soil and rate of soil respiration estimated with equation (2).

Phosphatase assay

Two 16-milliliter scintillation vials were obtained for each replicate of each treatment and labelled treatment or control. Vials were wrapped in aluminum foil to reduce exposure to sunlight. One gram of soil for each replicate was weighed into each treatment and control vials and 4 milliliters of Tris-malleate buffer (pH 7.0) added to each vial. Vials were gently swirled, and reaction initiated by adding 1 milliliter of 100 mM p-nitrophenylphosphate to treatment vials. Treatment and control vials were placed upright in an aluminum rack on a rotary shaker and shaken at 175 rpm for half hour. After shaking, 1 milliliter of 100 mM p-nitrophenylphosphate was added to control vials. Content of each vial was transferred into separate 16 milliliter centrifuge tubes and centrifuged at 10,000 rpm at 4°C for 10 minutes. The absorbance of the supernatant in each vial was analyzed with a spectrophotometer set at 400 nm. When absorbance was too high to detect, samples were diluted to 1:25. Standard curves were derived by preparing standard solutions ranging from 0 to 7.5 µM p-nitrophenol in tris-malleate buffer. Linear equations derived from standard curves with $R \geq 0.9905$ were used to calculate phosphate concentration in each vial (µmol phosphate L⁻¹) because one mole of each p-

nitrophenol produces one mole of phosphate. Phosphatase activity ($\mu\text{mol Pi evolved g}^{-1} \text{ h}^{-1}$) was estimated by first taking the difference between the phosphate concentration in treatment and control vials and using equation (4).

Urease assay

Two biplates were obtained for each replicate of each treatment and labelled test or control. Plates were placed on moist paper towel to minimize static electricity. One gram of soil from each of four replicates was weighed into one side of each biplate and 3 milliliters of 0.5 M Tris-malleate buffer (pH 7.0) with 1% sodium azide was pipetted into the soil-containing compartment of the biplate. Three milliliters of 2% boric acid indicator solution was pipetted into the other compartment of each biplate. One milliliter of 6-M urea solution was added to the soil and buffer solution in each replicate of each treatment. The same compartment of each control plate received one milliliter of distilled water to account for release of NH_3 from background NH_4^+ . Biplates were allowed to incubate at room temperature for one hour after which 0.5 milliliters of 10 mM AgSO_4 solution and one milliliter of 3-M K_2CO_3 added to terminate urease activity and release evolved NH_3 into the boric acid trap. Biplates were secured in Ziploc bags with plates of the separate treatments going into separate bags and allowed to incubate for 24 hours. Plates were carefully removed from bags 2 hours after incubation and titrated using 0.02-N HCl. Urease activity for each replicate of each treatment was calculated by applying equation (3).

Data Analysis

All statistical analyses were performed using JMP Pro 13. Each treatment was analyzed in replicates of four and values averaged. Treatment- time interaction effects were examined by

one-way analysis of variance (ANOVA). Normality assumptions were tested and differences between treatment means and controls were evaluated with Dunnet's test with $\alpha = 0.05$

RESULTS AND DISCUSSION

Soil respiration

Enhanced rate of soil respiration is indicative of high rate of microbial breakdown of carbon sources. This could be as a result of alternative sources of carbon from the application of these treatments or the activation of inactive soil microbes following the application of these treatments or both. Vivax and Magnus have high carbon contents (Table 3.1) which might be easily degradable and hence the high rate of soil respiration observed following their application. The carbon sources in these treatments might have been bound in such a way that they are easily degraded by soil microbes. This observation correlates to other observations in literature (Marinari et al., 2000; Tejada et al., 2009) which reported that supply of energy and nutrient sources stimulate soil microbial activity. Also, the enhanced rate of soil respiration could be attributed to the capacity of the products to activate the growth of soil microbes that were hitherto dormant. Rate of soil respiration for soil that received wetting agent application for 7 weeks ranged from 0.46 to 0.81 mg CO₂ g⁻¹.

Rate of soil respiration was neither stimulated nor suppressed by the application of these BPs. Kapre Reme- NSL and Kapre Reme-NSP have very low carbon contents (Table 3.2) suggesting that their application might not have served as carbon sources for soil microbes. Plant Helper has a relatively high carbon among content among BPs but its impact on rate of soil respiration was not significantly different from the control. This might be due to carbon in Plant Helper being bound in a manner that is not easily degradable.

When rates of WAs were compared with the Control, rates for Vivax, Fleet, Magnus, and Dispatch were significantly higher (Table 4.1), indicating that these products enhanced rate of soil respiration. This result suggests that the application of Vivax, Fleet, Magnus, and Dispatch might have acted as carbon sources for soil microbes and hence improved rate of soil respiration. To the contrary, Pervade, Duplex, Cascade, Sixteen 90, and Revolution did not significantly impact rate of soil respiration 7 weeks after treatment. At 14 WAT, Cascade and Sixteen 90 significantly suppressed rate of soil respiration (Table 4.1).

None of BPs significantly impacted rate of soil respiration 7 WAT (Figure 4.2). Among the PGRs investigated during the study, when rates were compared with the control, Cutless, Trimmit, and Proxy did not significantly impact rate of soil respiration 7 WAT while Anuew suppressed rate of soil respiration. (Table 4.3). Rate of soil respiration for BPs 7 WAT ranged from 0.43 to 0.47 mg CO₂ g⁻¹.

Urease Activity

The observation at 7 WAT demonstrate that the WAs could not stimulate or suppress microbial mediated conversion of urea sources in the soil to ammonia. This is presumably due to the very low nitrogen content in these products (Table 3.1). Another plausible explanation for this observation is that the application of these products could not cause the release of urease enzymes that may have been complexed to soil organic constituents. The increase in urease activity over time for Duplex and Revolution could be attributed to the possible release of bound urease enzyme following the application of these products. This suggests a slow impact of the WAs on the release of urease enzyme in soil due to the stable nature of the enzyme. Also, these products might have stimulated microbial activity increasing the production of urease enzyme.

Urease activity for Duplex and Revolution 14 WAT respectively increased by approximately 3.5 and 4 times the rates 7 WAT. This suggests that Duplex and Revolution liberated nitrogen sources over time leading to enhanced urease activity over time. Urease activity tends to increase with increasing organic matter content so the spike in urease activity particularly for Revolution could be as a result of increased organic matter caused by the high carbon content (Table 3.1) of this product. Although Duplex does not have as high carbon content as Revolution, the increased rate of urease activity for this treatment is likely that, it might have impacted urease activity by the increased availability of water following the application of this product.

Urease activity of pots treated with WAs for 7 weeks ranged from 1.5 to 4.0 $\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{h}^{-1}$. The highest and least rates of urease activity were recorded for Dispatch and Sixteen 90 respectively. There was no significant difference in mean urease activity for any treatment when compared with mean urease activity of the Control (Table 4.2). Mean urease activity of soil treated with WAs were higher at 14 WAT. Mean urease activity for all treatments increased at 14 WAT except Fleet. When rates at 14 WAT were compared with mean rate of the Control, Duplex and Revolution were significantly higher, indicating that these treatments enhanced rate of urease activity (Table 4.2). Rates for Vivax, Fleet, Dispatch, and Magnus were significantly lower than the control, indicating suppressive impact on urease activity. Cascade, Pervade, and Sixteen 90 did not significantly impact urease activity at 14 WAT.

Urease activity of PGRs at 7 WAT ranged from 2 to 4 $\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{h}^{-1}$ (Table 4.4). The highest mean rate of urease activity was recorded for Anuew. When rates were compared with the Control, there was no significant difference between Anuew and the Control. Mean rate of urease activity for Cutless, Trimmit, and Proxy were significantly lower than mean rate of the Control. This indicates that Cutless, Trimmit, and Proxy were inhibitory to urease

activity at 7 WAT. Although rate of urease activity for all PGRs increased, when rates were compared with the Control, none was significantly different at 14 WAT. A plausible reason for this observation is that the PGRs could not cause increased production of urease hence the lack of significant impact.

Urease activity recorded for BPs at 7 WAT ranged from 1.5 to 2 $\mu\text{mol NH}_3 \text{ evolved g}^{-1} \text{ h}^{-1}$ (Table 4.5). When rates were compared with the Control, there was no significant difference. This indicates that 7 WAT, urease activity was neither stimulated nor suppressed by the biological products. Urease activity at 14 WAT were higher than rates at 7 WAT although when rates 14 WAT were compared with the Control, there was no significant difference (Table 4.5). This high urease activity observed at 14 WAT could be attributed to the accumulation of substrate from the continued application of the product. This explanation agrees with findings of Huang et al. (1992) which reported that the activity of urease is substrate dependent, increasing with increased substrate concentration to reach a maximum. An alternative explanation for this observation is that the capacity of these products to stimulate ureolytic microbes to release urease is slow, hence urease is released at slow rate following the application of these products.

Phosphatase activity

Treatment-time interaction of treatments of BP, WAs, and PGRs was not significant for phosphatase activity. When treatments were compared with the Control, none of BPs or PGRs significantly impacted phosphatase activity (Figure 4.1) at 7 or 14 WAT. Mean rates of phosphatase activity for the 14-week duration ranged from 7.63 $\mu\text{mol Pi g}^{-1} \text{ h}^{-1}$ to 15.5 $\mu\text{mol Pi g}^{-1} \text{ h}^{-1}$. Rate of phosphatase activity was significantly different from the Control only for Cascade among all treatments. Cascade's rate was significantly higher than the Control, indicating that this treatment stimulated phosphatase activity (Figure 4.1).

Dollar spot severity as affected by wetting agents, plant growth regulators, and biological products.

There was no significant treatment time-interaction on severity of dollar spot (Table 4.6). When disease severity was compared with the Control, none of the treatments significantly suppressed severity of Dollar spot disease (Figure 4.3). This indicates that none of the treatments (WAs, PGRs, and BPs) significantly enhanced the disease suppressive nature of the soil. This suggests that the application of WAs, PGRs, and BPs did not improve the disease suppressive nature of the soil so as to suppress the severity of dollar spot disease. This agrees with the findings of Burpee et al. (1996) which concluded that the ability of PGRs to stimulate plant growth is not as significant as its impact on limiting disease severity.

Turfgrass Quality

Significant differences in turf quality (TQ) among wetting agent treatments occurred at 7 WAT (Table 4.7). Vivax, Fleet, Magnus, and Dispatch did not impact TQ at 7 WAT while Revolution, Cascade, Pervade, and Sixteen 90 significantly did. The improved turf quality following the application of Revolution, Cascade, Pervade and Sixteen 90 suggest that these treatments caused more wetting of the soil surface for water to infiltrate, improving moisture and hence TQ. Fay and Schultz (2009) reported improved growth of grass at greater soil moisture. TQ for turfgrass that were treated with Magnus, Dispatch, and Fleet were higher at 14 WAT than they were at 7 WAT. To the contrary, TQ for grass that was sprayed with Vivax, Sixteen 90, Revolution, Cascade, Pervade, and Duplex were lower at 14 WAT than they were at 7 WAT (Table 4.7). When TQ at 14 WAT was compared with the Control, none of the treatments significantly improved turf quality.

TQ for grass that was sprayed with PGRs at 7 WAT was highest for Proxy and Anuew and lowest for Cutless. However, TQ was compared with the Control, none of the PGRs significantly improved turf quality at 7 WAT (Table 4.8). TQ for grass treated with PGRs were all higher at 14 WAT than at 7 WAT but none were significantly different from the Control (Table 4.8). Although there was no significant improvement in quality of grass when compared with the control, quality ratings of grass increased with continued application.

TQ for all BPs improved over time. However, when ratings were compared with Control, none were significantly different at 14 WAT (Table 4.8). Kapre Reme NSL had the highest TQ 7 WAT. When ratings were compared with Control, Kapre Reme NSL significantly improved turf quality (Table 4.9).

Overall, the products that significantly improved TQ did so in the early stages of the study (at 7 WAT). TQ improved over time except for Sixteen 90 and Cascade. The improved TQ over time following the application of products agrees with the findings of Karnok and Tucker (2001b) which reported improved turfgrass color and quality with time following the application of WAs.

Table 4.1. Impact of wetting agents applied to a mixture of sand/peat moss (90:10) at the recommended rate on rate of soil respiration in a 7 and 14-week Greenhouse study. CO₂ emitted at each sampling time is compared against their respective Control.

Treatment	7 WAT (mg CO ₂ g ⁻¹)	14 WAT (mg CO ₂ g ⁻¹)
Vivax	0.80a	20.0a
Fleet	0.80a	19.5a
Magnus	0.82a	20.4a
Dispatch	0.77a	19.3a
Pervade	0.52b	19.6a
Revolution	0.46b	20.2a
Sixteen 90	0.47b	6.6b
Cascade	0.47b	7.3b
Duplex	0.52b	20.1a
Control	0.53b	20.5a

¹WAT = Weeks after treatment. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's test.

Table 4.2. Impact of wetting agents applied to a mixture of sand/peat moss (90:10) at the recommended rate on urease activity ($\mu\text{mol NH}_3 \text{ evolved g}^{-1} \text{ h}^{-1}$) in a 7 and 14-week Greenhouse study. $\mu\text{mol NH}_3 \text{ evolved g}^{-1} \text{ h}^{-1}$ at each sampling time is compared against their respective Control.

Treatment	7 WAT ($\mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$)	14 WAT ($\mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$)
Vivax	2.5a	4.0b
Fleet	3.5a	3.5b
Magnus	2.5a	5.0b
Dispatch	3.5a	4.5b
Pervade	2.0a	8.5b
Revolution	2.5a	10.0a
Sixteen 90	1.5a	8.5a
Cascade	2.5a	9.5a
Duplex	3.5a	10.0a
Control	3.0a	8.0b

¹WAT = Weeks after treatment. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's

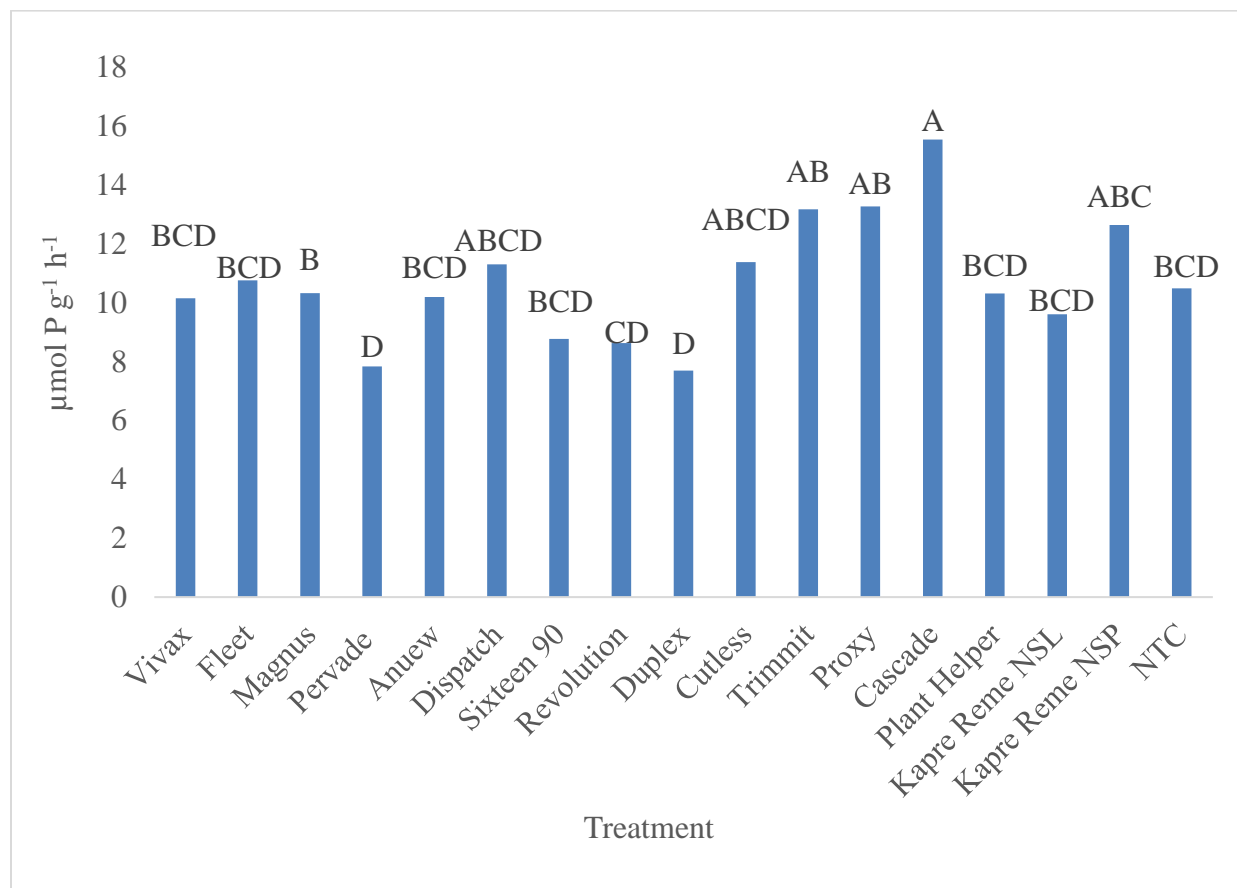


Figure 4.1. Mean impact of wetting agents, plant growth regulators, and biological products on phosphatase activity 7 and 14 weeks after application. Treatments were applied at the recommended application rate and phosphatase activity determined colorimetrically.

Table 4.3. Impact of plant growth regulators applied to a mixture of sand/peat moss (90:10) at the recommended rate on rate of soil respiration in a 7 and 14-week Greenhouse study. CO₂ emitted at each sampling time is compared against their respective Control.

Treatment	7 WAT (mg CO ₂ g ⁻¹)	14 WAT (mg CO ₂ g ⁻¹)
Cutless	0.62a	¹⁾
Trimmit	0.61a	0.52b
Proxy	0.59a	0.35b
Anuew	0.38b	0.84 a
Control	0.60a	0.15b
		0.82a

Table 4.4. Impact of plant growth regulators applied to a mixture of sand/peat moss (90:10) at the recommended rate on urease activity ($\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{h}^{-1}$) in a 7 and 14-week Greenhouse study. $\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{h}^{-1}$ at each sampling time is compared against their respective Control.

Treatment	7 WAT ($\mu\text{mol NH}_3 \text{g}^{-1}\text{h}^{-1}$)	14 WAT ($\mu\text{mol NH}_3 \text{g}^{-1}\text{h}^{-1}$)
Trimmit	2.0a	5.0a
Cutless	2.0a	4.0a
Anuew	3.0a	5.0a
Proxy	2.0a	4.5a
Control	4.0a	4.0a

¹WAT = Weeks after treatment. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's

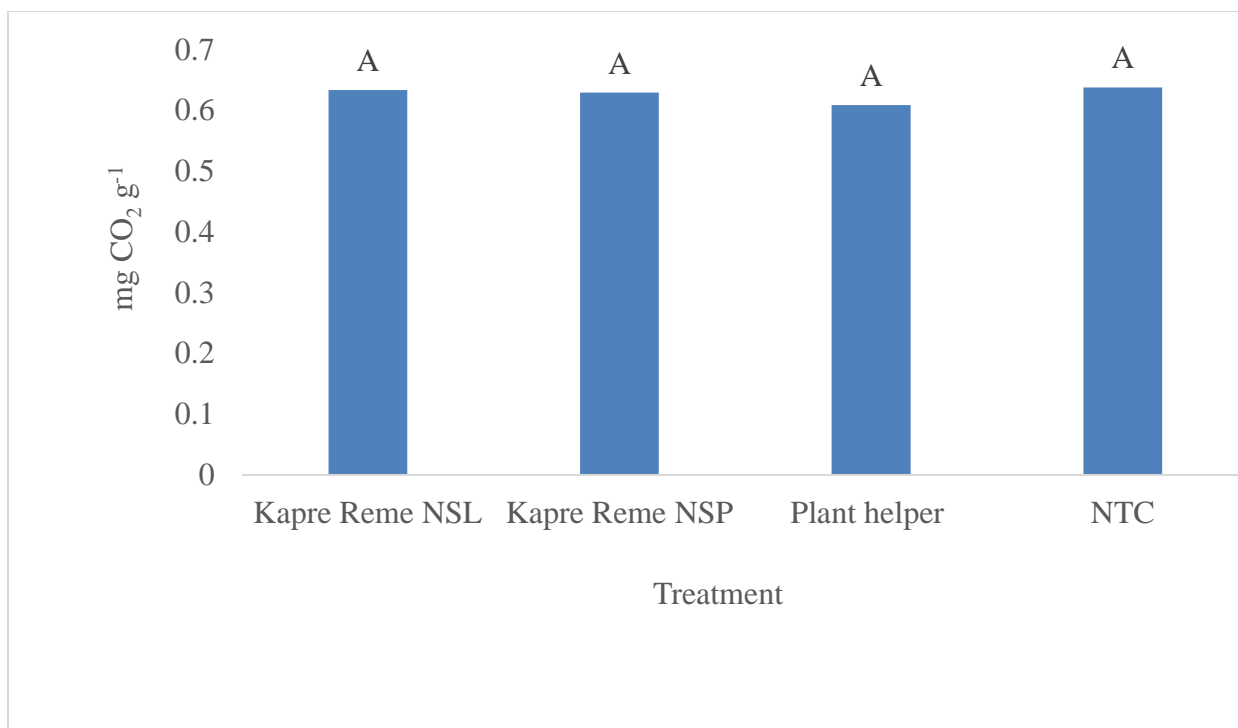


Figure 4.2 Mean impact of biological products on rate of soil respiration at 7 and 14 weeks after treatment application. Treatment were applied at the recommended rates and rate of soil respiration determined by the alkaline trap method.

Table 4.5. Impact of biological products applied to a mixture of sand/peat moss (90:10) at the recommended rate on urease activity ($\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{h}^{-1}$) in a 7 and 14-week Greenhouse study. $\mu\text{mol NH}_3$ evolved $\text{g}^{-1} \text{h}^{-1}$ at each sampling time is compared against their respective Control.

Treatment	7 WAT ($\mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$)	14 WAT ($\mu\text{mol NH}_3 \text{ g}^{-1} \text{ h}^{-1}$)
Plant Helper	2.0a	6.5a
Kapre Reme NSL	1.5a	10.0a
Kapre Reme NSP	1.5a	8.0a
Control	2.0a	8.0a

¹WAT = Weeks after treatment. Means in the same column joined by the same letter are not significantly different at the 0.05 level of probability according to Dunnett's

Table 4.6. One-way ANOVA of treatment effect on severity of dollar spot 10, 20, 30, and 40 days after inoculation.

	DF	Sum of squares	F Ratio	Prob > F
Treatment	15	1431.9957	1.6590	0.1123
Time	3	213.4717	3.7097	0.0630
Treatment * time	45	383.5758	0.4513	0.9481

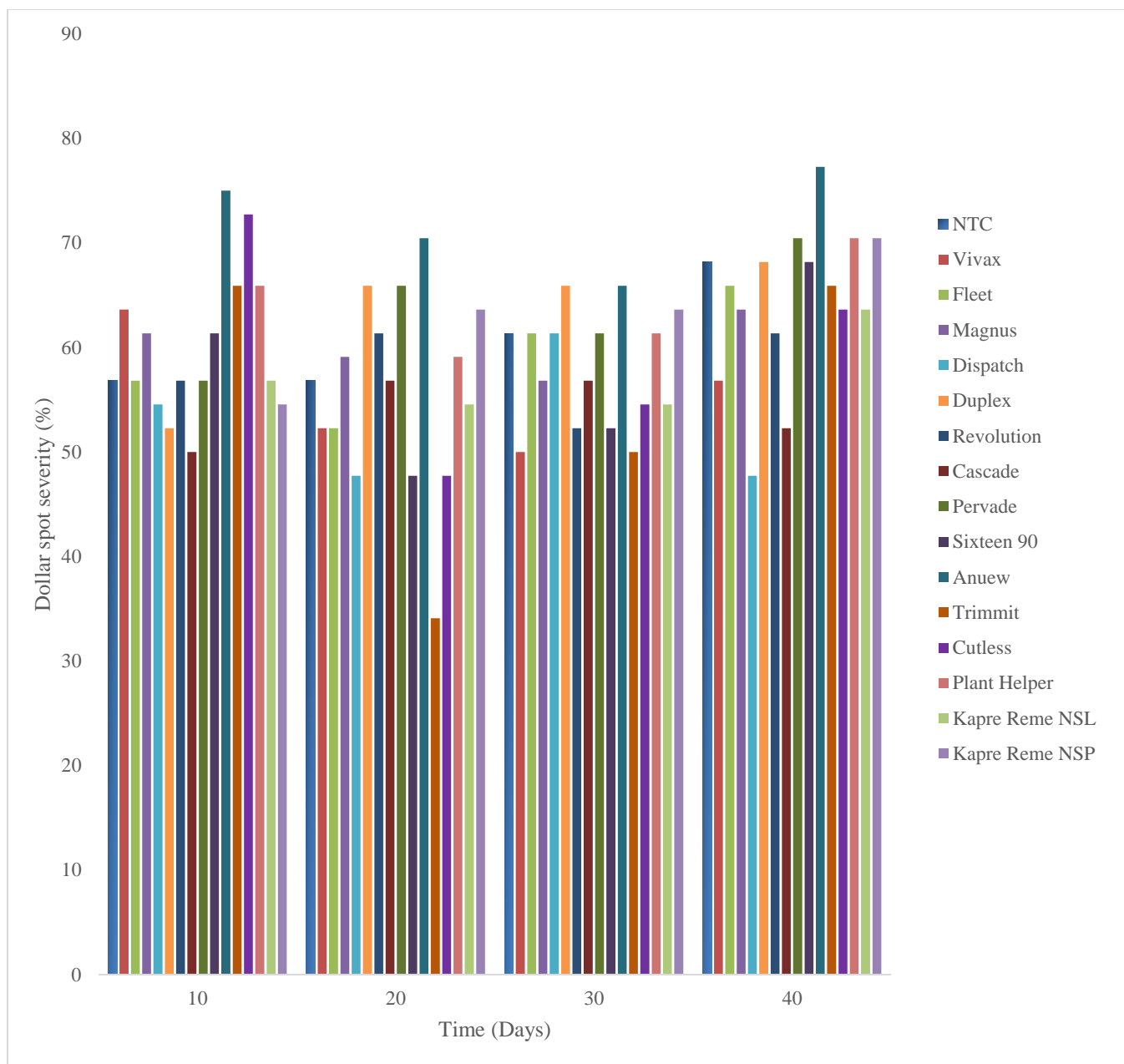


Figure 4.3. Dollar spot severity on bentgrass as impacted by wetting agents, plant growth regulators, and biological products at 10, 20, 30, and 40 days after inoculation in the Greenhouse. Severity was rated on a scale of 1 to 11 with 1 being the least infected center and 11 the worst infected.

Table 4.7. NDVI of bentgrass as affected by wetting agents at 7 and 14 weeks after treatment application. Images were analyzed with FieldAnalyzer software.

Treatment	7 WAT	14 WAT
Vivax	0.69a	0.59a
Fleet	0.64a	0.66a
Magnus	0.71a	0.62a
Dispatch	0.64a	0.61a
Control	0.66a	0.61a
Pervade	0.79a	0.70a
Revolution	0.80a	0.70a
Sixteen 90	0.78a	0.69a
Cascade	0.86a	0.73a
Duplex	0.82a	0.71a
Control	0.61b	0.65a

¹WAT = Weeks after treatment.

²Values in the same column separated by different letters are significantly different at the 0.05 level of probability according to Dunnett's test. Comparison is valid between control and other treatments.

Table 4.8. NDVI of bentgrass as affected by plant growth regulators at 7 and 14 weeks after treatment application. Images were analyzed with FieldAnalyzer software.

Treatment	7 WAT	14 WAT
Cutless	0.59a	0.62a
Trimmit	0.61a	0.64a
Proxy	0.62a	0.58a
Anuew	0.65a	0.60a
Control	0.65a	0.57a

¹WAT = Weeks after treatment.

²Values in the same column separated by different letters are significantly different at the 0.05 level of probability according to Dunnett's test. Comparison is valid between control and other treatments.

Table 4.9. NDVI of bentgrass as affected by biological products at 7 and 14 weeks after treatment application. Images were taken with light tube and analyzed with FieldAnalyzer software

Treatment	7 WAT	14 WAT
Plant helper	0.54b	0.51b
Kapre Reme NSL	0.51a	0.53a
Kapre Reme NSP	0.49b	0.56a
Control	0.52b	0.54a

¹WAT = Weeks after treatment.

²Values in the same column separated by different letters are significantly different at the 0.05 level of probability according to Dunnett's test. Comparison is valid between control and other treatments.

CONCLUSIONS

None of the WAs significantly impacted urease activity 7 at WAT. Duplex and Revolution enhanced urease activity at 14 WAT whereas Magnus and Dispatch suppressed urease activity 14 WAT. Impact of PGRs on urease activity also fluctuated as was observed with the WAs. Cutless, Trimmit, and Proxy suppressed urease activity at 7 WAT. AT 14 WAT, activity of urease increased for the PGRs but when compared with the Control, there was no significant impact. None of the BPs impacted urease activity at 7 WAT. Urease activity for BPs increased at 14 WAT but were not significantly different from the control. Phosphatase was impacted only by Cascade. Soil respiration was significantly enhanced by Vivax, Fleet and Magnus at 7 WAT. However, there was no significant impact of WAs on soil respiration at 14 WAT. None of the BPs significantly impacted soil respiration during the duration of the study. Among the PGRs, Anuew had a suppressing impact on soil respiration both at 7 and 14 WAT while Cutless suppressed soil respiration at 14 WAT.

Turf quality was improved by Pervade, Revolution, Sixteen 90, and Cascade at 7 WAT. At 14 WAT, no WAs improved turf quality. None of the PGRs significantly improved turf quality for the duration of the study while Kapre Reme NSL, a BP improved turf quality at 7 WAT. Disease suppressive nature of the soil was not improved as inferred from the lack of suppression of Dollar spot disease over time following the application of treatments.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

This study gave insight to the long-term impact of turf care products on soil biological health. In the Laboratory study, where products were applied at 100 x the recommended application rates, the impact of the products on soil biological health over a long period was simulated. Some of the products enhanced the variables of this study while others had suppressive impacts. In some cases, variables were neither suppressed nor enhanced. Among wetting agents, Sixteen 90 and Duplex enhanced rate of soil respiration while this was suppressed by Vivax and Magnus. Vivax, Fleet, Magnus, and Sixteen 90 enhanced urease activity while phosphatase activity was suppressed by Dispatch. Among PGRs, Anuew, Cutless, and Proxy enhanced soil respiration, phosphatase activity, and urease activity respectively. Biological products neither enhanced nor suppressed the variables of our study.

In the Greenhouse study where treatments were applied at the recommended application rates, Vivax, Fleet, Magnus, and Dispatch enhanced rate of soil respiration 7 weeks after treatment application while urease activity was not impacted by any wetting agent. Cascade enhanced phosphatase activity in the greenhouse study. Turf quality was improved by the application of some treatments. Revolution, Sixteen 90, Cascade, and Duplex improved turf quality 7 weeks after treatment application. Turf quality was not improved by any plant growth regulator while Kapre Reme NSL, a biological product, improved turf quality. Disease suppressive nature of the soil was not improved by the application of treatments as evidenced by the lack of suppression of Dollar spot progression in the Greenhouse study.