EVALUATING THE IMPACT OF OXYGENATED NANOBUBBLE WATER ON TURFGRASS GROWTH AND SOIL BIOLOGICAL HEALTH

by

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(Under the Direction of Mussie Y. Habteselassie)

ABSTRACT

Research was conducted at a golf course in Johns Creek, GA from August 2020 to August 2022 and a greenhouse in Griffin, GA for 3 years to evaluate the effects of oxygenated nanobubble water on microbial abundance and function, turf quality and growth, and water movement and retention. Measured response parameters included bacterial and fungal abundance, enzyme activities and soil respiration, shoot and root growth, turf quality, and infiltration rate. Soil oxygen and temperature were continuously monitored with in-situ sensors. The oxygenated nanobubble water did not show consistently significant effects on turf quality, shoot, root growth, or microbial abundance and function in either greenhouse or field trials. For example, root weight was significantly higher for treatment in the field in 2022, but not in 2021. However, oxygenated nanobubble water impaired infiltration rates in the greenhouse trials. Overall, oxygenated nanobubble water did not impact turf or soil health parameters.

INDEX WORDS: microbial abundance, turf quality, oxygenated nanobubble water, enzyme activity, soil respiration, infiltration rate, relative leaf water index

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CHAPTER 1

INTRODUCTION

The turfgrass industry has changed in many ways in the past two decades, including turf equipment used, seed and sod, turf quality, new active chemicals, and turf verities (Johnson et al., 2015). As there is an increase in urbanization and recreation with the paralleled increase in population, there is a growing need for turfgrass and its utilization in golf courses, sporting fields, and public parks. Approximately 60% of urban land that is expected to exist by 2030 is being built between 2000 and 2030 (Elmqvist et al., 2015). Urban lawns, using turfgrasses, are the city's most common part of open spaces and green infrastructure (Castilho et al., 2020).

With the inclusion of golf courses, parks, and home lawns, turfgrass covers 14% of land in the United States (Yao et al., 2006). There is a strong demand for residential and commercial property development and the environmental and aesthetic benefits associated with the use of turfgrass (Haydu et al., 2008). This demand for aesthetic turfgrass applies to golf course management. A study published in 1993 showed that over 24 million golfers play 500 million rounds of golf on over 15,000 golf courses in the United States (Beard & Green, 1993). The golf industry has remained stable since then and even found that younger generations are participating with 36% of the 24 million being between the ages of 19 to 39 (Spilman, 2019). It has also been found that the golf sector accounts for 44% of the turfgrass industry and that United States golf courses generated $33.2 billion in gross economic impacts (Johnson et al., 2015). Golf course maintenance embodies many of the challenges involved in turfgrass production, including increased pressure in the optimized use of resources, reduction of chemical inputs and pesticide inputs, golf course compaction and soil oxygen deficiency, and low water use efficiency (Johnson et al., 2015; Strandberg et al., 2012) Many of these aesthetic and environmental pressures are placed on superintendents to upkeep quality turfgrass. These resource inputs can include fertilizers, pesticides, water, growth regulators, and herbicides, driving a very environmentally taxing, as well as costly, agricultural system. From this pull of resources and money and changing attitudes, superintendents are reconsidering the management of their courses, attempting to be more efficient in the use of their resources, reducing the costs and quantities of their inputs, and lessening their environmental impacts (Strandberg et al., 2012). To address some of these concerns, for water irrigation and soil health, golf course superintendents are experimenting with new technology, one of which being nanobubble technology.

Nanobubbles are gaseous particles that can be attached to a surface or dispersed within a liquid. As they are small in size (being below 1000nm – a millionth of a meter in diameter), they have a large surface area per unit volume, getting a concentration as high as a hundred million to ten trillion bubbles per millimeter of liquid (Atkinson et al., 2019). Nanobubbles are negatively charged in the pH range that is common in the environment (2 to 12) (Temesgen, 2017). In addition, because of nanobubbles' extremely low rising velocity and their extremely high surface area per unit volume, they are stable in liquid for an extended period, in some cases up to several weeks (Atkinson et al., 2019; Kalogerakis et al., 2021). The techniques for the generation of nanobubbles are wide, including cavity formation by creating pressure differences below a certain critical value that promotes cavitation, sonication, electrolysis, and the use of a membrane to push gases of certain sizes into a flowing liquid (membrane method) (Phan et al., 2020). Several gases have been used in combination with the membrane method to produce nanobubbles, the gas most common being oxygen to produce oxygenated nanobubble water. Through the rupture and collapse of oxygenated nanobubbles, reactive oxygen species (ROS), such as peroxides and hydroxyl radicals, have been created in response (Atkinson et al., 2019). The infusion of oxygenated nanobubbles into irrigation water can have several potential applications in a turfgrass system and help address some of the challenges described above.

One application is the potential in improving aeration in a turfgrass root system. Oxygenated nanobubble water has the potential to improve oxygen availability, improving root performance, water use efficiency, and biomass, as well as stimulate the activity of microorganisms in the rootzone responsible for the decomposition of organic matter (Zhu et al., 2019; Pendergast et al., 2013; Lei et al., 2016). Another application is the potential impact of nanobubbles on water movement and retention in the soil. This potential impact is because of the effect oxygenated nanobubbles have on surface tension, as well as this technology being reported as acting as a bridging agent bringing together hydrophobic surfaces by influencing surface forces (Alheshibri et al., 2016). This may reduce water repellency in turfgrass soils caused by hydrophobic surfaces. In addition, oxygenated nanobubble water may influence water use rates in plants, furthering the influence this technology has on improving irrigation practices (Yaxin Liu et al., 2019). Another application is the use of oxygenated nanobubble water in pathogen control, with the production of ROS, showing an antimicrobial effect, suppressing common turfgrass diseases, but also potentially killing microbial communities responsible for nutrient cycling, soil organic matter production, and disease suppression (Schlatter et al., 2017; Liang et al., 2018; Myrold and Bottomley et al., 2008).

Research is needed to understand how oxygenated nanobubble water affects soil microbial communities, which play a role in establishing and maintaining a healthy and thriving turfgrass ecosystem. It is, therefore, logical to ask: can we enhance the beneficial roles of soil microorganisms and aeration of the soil to decrease external inputs and maintain a healthy and sustainable turfgrass system? This requires a proper understanding of the impact of oxygenated nanobubble water on the soil microbial community and turf growth and quality.

Significance of the Study

The key research question we want to address in this study is: does oxygenated nanobubble water positively impact the turfgrass soil microbial communities, turfgrass growth, and quality, as well as water movement and retention? This research question is important to address as it will determine how oxygenated nanobubbles can impact organic matter decomposition, nutrient acquisition and availability, and disease suppression, which are important for turfgrass growth and quality in golf courses. If any of these functions are positively impacted, there will be financial benefits to superintendents due to reduced inputs of commercial products, making the industry more sustainable. Our objectives are to examine the impact that oxygenated nanobubbles have on turf growth and quality, water movement and retention, and soil microbial activity and abundance in the greenhouse and field.

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CHAPTER 2

LITERATURE REVIEW

Growth of the turfgrass and golfing industry in the USA

Dating back to historical gardens in Asia and Europe, to the early research conducted in the Olcott Turf Gardens in Connecticut, to the modern applications of turfgrass use on golf courses and in American suburbia, this ornamental has intertwined itself with human history and culture (Roberts et al., 1992). In the United States, turfgrass reaches approximately 62 million acres of maintained land, including lawns, parks, golf courses, and highways, with the turfgrass industry being a $90 billion industry in the year 2000 (Chawla et al., 2018). In more relative terms to the state of Georgia, turfgrass and the related industry contributes $7.8 billion to Georgia's over 700-billion-dollar economy and account for a total of nearly 87,000 full and part-time jobs (Kane & Wolfe, 2012). There are also indirect benefits of the turfgrass industry through the supply chains encompassed in this market. Turfgrass provides economic and employment benefits in the sod production industry, lawn equipment manufacturing industry, lawncare goods retailing sector, and lawncare service sector. In addition, the golf course industry is a 37.8-billion-dollar industry that supplies over 360,000 jobs (Haydu et al., 2006). With golf courses being at the forefront of the development of the turfgrass industry, the growth of turfgrass in residential and public recreational spaces has increased in recent years. With the expansion of the golfing industry, the need for resource inputs, chemical inputs, and maintenance increases as well.

Urbanization and its relation to turfgrass

Healthy agricultural soil has the capability of supporting the production of a desired crop or ornamental to high-quality standards, being sufficient to meet human requirements and maintain those standards that are essential to maintain the quality of life for humans as well as the conservation of biodiversity (Singh, 2018). More specifically in this definition is the maintenance of the quality of life for humans. Through the act of urbanization, turfgrass has become a staple in its use and incorporation into green spaces in a modernizing world. However, because of urbanization, there are problems affecting turfgrass communities. These produce problems of water availability and oversaturation in the soils of turfgrasses. In addition, anthropogenic traffic can influence soil's physical properties. Consistent traffic has the potential to alter bulk density, turf strength, aeration, moisture, temperature, and infiltration. More specifically, previous research focuses heavily on how traffic-induced compaction has resulted in a reduced amount of soil oxygen and led to decreased turf growth and increased competition with weeds (Steinke & Ervin, 2015). Turfgrass maintenance through mowing and additives have led turf areas to be both a source and sink for carbon dioxide, methane, nitrous oxide, nitrogen oxides, and non-greenhouse-gas pollutants (Stier et al., 2015). It was previously found that urban lawns consume 5% of atmospheric methane, while also emitting 30% of nitrous oxide (a gas 300 times more potent than carbon dioxide and can deplete the ozone layer) (Stier et al., 2015). This report shows that even though there are benefits to using turfgrass to combat climate change, the reduction of inputs into the turfgrass industry is significant in alleviating human impacts on our world. Another anthropogenic problem affecting the turfgrass industry is soil disturbance. Within soil disturbance are compaction, changes in plant community dynamics, decreased biodiversity, reduced microbial succession, and altering of the food web of the site (Steinke & Ervin, 2015).

For instance, with the addition of fertilizers to the soil, there has been a reduced uniformity of microbial species due to a limited number of species benefitting from excess nutrient levels which can disrupt the oligotrophic state the site was originally in (Van Bruggen & Semenov, 2000). In addition, disturbances, like chemical application and tillage, can alter seed availability, open and close different soil community niches, and can alter plant succession patterns (Sheley, 2005). Anthropogenic disturbances, whether they be traffic, compaction and soil disturbance, fossil fuel pollutants, chemical additives, or irrigation patterns may reduce below-ground biodiversity through site alterations and long-term land use. Reducing overall inputs into the turfgrass industry is important in promoting sustainable turfgrass management.

Pesticide and fertilizer inputs in the turfgrass industry

Pesticides are an input and a concern in turfgrass systems, as they can be a risk to human health and may indirectly contaminate the groundwater to which they are applied. Although the thatch layer, an organic accumulation of dead and living roots and stems nestled between the canopy and the soil surface, is important for golf playability, too thick of a thatch layer can cause reduced water filtration, shallow rooting, and increased vulnerability to pest problems and stress (Couillard & Turgeon, 1997). This thatch accumulation can be caused by the application of pesticides that kill earthworms and soil microbes that are important to the decomposition process of this layer (Potter &Braman, 1991). The extinguishment of earthworms by pesticides can also lead to soil compaction and reduced air circulation in the soils, indirectly killing plants due to oxygen depletion in the soil or resulting in costly technology to reacquire more optimal soil conditions (Potter &Braman, 1991).

Through the application of inorganic chemical fertilizers, there is acidification of the soil, soil layer compaction, oversaturation of the soil, and oxygen deficiencies in soil layers (Bitew & Alemayehu, 2017). In addition, the overuse of pesticides, herbicides, and fungicides, has been found to disrupt nutrient cycling conducted by soil microbes. For instance, chlorothalonil and dinitrophenyl fungicides show disruption of nitrification and denitrification bacteria-dependent processes (Mahmood et al., 2016). The herbicide triclopyr inhibits soil bacteria that are involved in the transformation of ammonia into nitrite (Mahmood et al., 2016). The non-selective herbicide Glyphosate reduces the growth and activity of nitrogen-fixing bacteria in the soil through 2,4-D that can be found in glyphosate inhibiting the transformation of ammonia into nitrate carried out by soil bacteria (Mahmood et al., 2016). Not only are soil microbial populations affected, but the use of chemical additives in the soil can affect fungal populations as well. The pesticides trifluralin and oryzalin are both known to inhibit the growth of symbiotic mycorrhizal fungi that help with nutrient uptake, as well as oxadiazon is known to reduce the number of fungal spores (Mahmood et al., 2016). As a result of the excessive applications of fertilizers, fungicides, and pesticides to achieve high-quality turfgrass, there are direct and indirect consequences of the use of chemical resources on the environment that affect the microbial biodiversity of the soil. The use of excessive inputs is not only harmful to the soil microbial population but is offsetting the good the turfgrasses can do for the environment. Turfgrass landscapes tend to have greater carbon stocks than a standard agricultural system, even approaching levels of a native forest (Thompson & Kao-Kniffin, 2017). This makes turfgrass a viable solution to adding carbon sequestration to urban landscapes. However, carbon emissions resulting from management practices (such as fossil fuels for mowing, energy for irrigation, and energy for fertilizer production and application) show a decrease or even a complete offset of this carbon storage in urban grasslands (Thompson & Kao-Kniffin, 2017). This shows that the current management practices of turfgrass are unsustainable and are counteracting the benefits it can do to urban spaces.

Golf courses consume a multitude of commercial chemicals and technological services (Chawla et al., 2018). Inputs can include fertilizers, pesticides, water, growth regulators, and herbicides, making it conceivable that these managed ecosystems may have less diverse microbial communities compared to their natural counterparts (Yao et al. 2006). These pressures to maintain quality turfgrass are placed on superintendents, motivating many of them to seek less environmentally harmful methods. From this pull of resources and money, superintendents are reconsidering the management of their courses, attempting to be more efficient in the use of their resources, reducing the costs and quantities of their inputs, and lessening their environmental impacts (Strandberg et al., 2012). As a result, there is motivation in the industry to preserve natural resources and soil biodiversity, as well as make the construction and management of future and existing golf courses more environmentally compatible (Terman, 1997).

Irrigation in the turfgrass industry

Irrigation plays a major role in the improvement of turfgrass quality and playability. Golf courses in the United States were found to use 1.44% of all irrigation water for the continent, with the total amount being 1.86 million acre-feet of water per year (Gelernter et al., 2015). More specifically in the Southeast, there have been efforts in water conservation with the highest decrease in mean water usage being in this region in a survey taken comparing usage from 2005 to 2013 (Gelernter et al., 2015). There has been rising competition for water between leisure, agriculture, and residential water supply that is driving the turfgrass sector to reduce water and energy consumption while also maintaining high turf quality (Gómez-Armayones et al., 2018). Because turfgrass is a perennial crop, furrowing and flooding are unsuitable, making sprinkler irrigation systems the preferred method for this ornamental (Bastug & Buyuktas, 2003). But, because of the push for sustainable practices in the turfgrass industry, stressing the turf has been explored as an option for reducing water. Being the most effective water conservation strategy in areas that receive sufficient rainfall, deficit irrigation is the practice of irrigating turfgrass stands at rates that are lower than the maximum water loss (Johnson et al., 2015). The imposition of deficit irrigation can be achieved by decreasing the amount of water in each irrigation event, increasing the time between irrigation events, or by decreasing irrigation events during less sensitive growing stages of the grasses (Johnson et al., 2015). Excessive drought can be a problem on courses being underwatered and can affect turfgrass through the visual quality, growth rate, playability, and evapotranspiration, so to maintain the appearance of the ornamental, proper irrigation is necessary (Diaz et al., 2007). Irrigation practices are an indicator of turfgrass quality. It has been found that in both warm-season and cold-season turfgrasses, visual turfgrass quality increased with higher irrigation, the best turf quality being at 100% evapotranspiration (Gómez-Armayones et al., 2018). However, an acceptable turfgrass quality is maintained at deficit irrigation, with a score of 6 or above being given to grasses with a deficit of above 40% evapotranspiration (Gómez-Armayones et al., 2018). In addition, turfgrass species can maintain quality and function, even when watered at mild drought-stress levels because of their drought resistance mechanisms and ability to successfully recover from longer-term water deficits. However, water use rates by turfgrasses can be affected by climatic conditions, soil type, soil water availability, water quality, growth habits, mowing height, fertility, and application of plant growth regulators (Johnson et al., 2015). Research into irrigation practices, although variable based on a multitude of factors, is important in finding better solutions to make turfgrass production more sustainable.

Thatch and soil compaction problems in turfgrass

Thatch in turfgrass has been described as a tight and intermingled layer of dead and living organic matter that develops between the green vegetation and the soil surface (Couillard & Turgeon, 1997). Thatch accumulates due to imbalances between the production of organic matter and the decomposition of organic matter, leading to any factor which causes the production of vegetative matter to exceed the decomposition rate to increase thatch production (Murray & Juska, 1977). A thin layer of thatch can be beneficial by enhancing the tolerance of turf and acting as a buffer against temperature extremes, moisture extremes, and chemical inputs (Couillard & Turgeon, 1997). The excessive accumulation of the thatch layer has adverse effects on the soil, including increased disease, increased insect problems, higher susceptibility to heat or cold stress, drought stresses, and greater soil compaction (Couillard & Turgeon, 1997). It has been found that an excessive layer of thatch can reduce water infiltration rates into sandy soils, while sand without excessive thatch showed higher infiltration rates (Taylor & Blake, 1982). A lower infiltration rate can result in the deterioration of the quality of turfgrass, the quality of play on a sporting field, stress on grasses, and the increased dangers of compaction and puddling of the soil (Taylor & Blake, 1982). Compacted soils have reduced spaces in soil that generally carry water and air (Kopp, 2011). As a result of compaction in soil, the industry requires techniques to aerate the turfgrass, some of which are core aerification, solid tine aerification, or the technique of dethatching with a vertical mower or power rake (Kopp, 2011). Although the best solution to reduce soil compaction is to reduce traffic on the sporting field, in many cases this solution is not viable. With new technologies improving the aerification process in turfgrasses through new fairway cultivation equipment, there is still a gap for equipment to streamline the verification process in getting oxygen into compacted soils (Murphy & Scott, 2015). Soil oxygen deficiency is a common problem in turfgrasses found in sporting fields.

Soil health and parameters for measuring soil health

Soil health has been defined as, “The continued capacity of soil to function as a vital living system, within an ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health” (Nielsen et al., 2002; Doran & Zeiss, 2000). “Soil health” and “soil quality” are synonymous terms and will be used interchangeably throughout this paper. This definition encompasses the idea of soil being a living system. Soil is essential to the maintenance of environmental quality, provides ecosystem functions, as well as are a primary step in environmental renewal and sustainability. Healthy management of soils can lead to the reduced need for agrochemicals and tillage, increased sequestration of carbon dioxide within the soil, and reduced fuel consumption by maintenance equipment (Brevick, 2010). Soils provide essential services like water purification, carbon sequestration, nutrient cycling, and being habitats for biodiversity, as well as being susceptible to agricultural management (Rinot et al., 2019).

Soil microbial health is one factor of soil biodiversity. Beneficial soil microorganisms provide a multitude of functions in an ecosystem, including nutrient capture and cycling, organic matter decomposition, soil organic matter dynamics, soil structure maintenance, biological population regulation, habitat provisions, and disease suppression (Kibblewhite et al., 2008). Some important indicators of biological soil health are abundance and composition, as well as their activity in soil systems through soil respiration and enzymatic activity (Janvier et al., 2007). Without the presence of biodiversity, the capacity of soil biota to deliver ecosystem services reduces, and resilience is lost (Kibblewhite et al., 2008). In measuring biodiversity in a soil system, it is best to distinguish the groups by microbial level, including bacteria, fungi, and ammonia-oxidizers, to reduce redundancy if they were to be characterized by soil microbial species (Van Bruggen & Semenov, 2000). These aspects play into the definition of soil health of sustaining biological productivity within a turfgrass system. It has been previously noted that diverse arrays of bacteria, including species of *Pseudomonas, Azospirillum, Azotobacter, and Bacillus*, promote overall plant growth and improve soil structure (Awan et al., 2011). Without functions taking place in soil by the soil microbes, any crop or ornamental would cease to survive. This makes soil microorganisms a viable measure of soil health.

Turfgrasses are typically grown on urban disturbed soil profiles, which are characterized by courser textures, elevated pH, high amounts of soluble salts, plant nutrients, and heavy metals, as compared to undisturbed counterparts (Murphy & Scott Ebdon, 2015). This contrasts with a healthy soil ecosystem that can recover from or even adapt to stress, the soil is mostly dependent on its biological component to give it this resiliency (Lehman et al., 2015). Just as there is diversity in other natural systems, there is diversity in soil microbial populations that all can supplement the benefits of a turfgrass system. One soil process is the nutrient cycling of nitrogen. Nitrogen fixation, the reduction of atmospheric N2 to bioavailable NH4, and biological nitrification (oxidation of NH4 to NO3-) are important in providing natural nitrogen inputs to plants (Visconti et al., 2020). These microbial populations are very sensitive to environmental change and stress and can be regarded as indicators of soil health. More specifically, in the study of N-cycling genes that are involved in N-transformations, N-fixation (*nif*H) and nitrification (*amo*A) have been used in assessing the abundance and diversity of soil microbial communities (Visconti et al., 2020).

It has been noted that the stability of a soil ecosystem is significantly linked to the abundance of different functional groups of soil microorganisms, with different organisms providing different energy and nutrient flow to the site (Barrios, 2007). With a slew of environmental factors (ie. pH, temperature, soil organic matter, soil-water content, etc.) influencing the nitrification process, the community of denitrifying and nitrifying bacteria varies in a soil ecosystem (Dell et al., 2010). Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) are two significant microorganisms involved in the autotrophic nitrification process. These microorganisms drive the first step of nitrification, ammonia oxidation (Sun et al., 2019). They are oligotrophic microorganisms with sturdy cellular structures and can survive in stressful soil conditions, such as nutrient-depleted or anoxic conditions. Microbial ammonia oxidation is the first and the rate-limiting step of nitrification, thus without AOA ad AOB assuming this role nitrification would not occur (Morimoto et al., 2011). It has been extensively researched that AOA is more dominant in terrestrial environments than AOB and that ammonia, pH, carbon sources, temperature, moisture, and oxygen can define the distinct ecological niches of AOA and AOB (Sun et al., 2019). AOA has been suggested to have an advantage over AOB in terms of adaptability of ammonium-nitrogen conditions (such as after nitrogen fertilizer application), but because of their physiological differences, AOA and AOB can coexist in the same soil despite both using ammonium as their primary energy source (Morimoto et al., 2011). Long-term fertilization and pH both play a significant role in the community composition of AOA and AOB (Sun et al., 2019). The diversity and community composition of these ammonia oxidizers act as indicators of soil health and quality, more specifically in systems with high rates of nitrogen-based fertilizer application (Zhou et al., 2022).

The investigation of enzymes shows how management practices affect their activities (Bandick & Dick, 1999). As new microbial and enzyme assays are discovered and become available, there is a stronger relationship produced between enzyme activities and soil biological parameters (Dick, 2015). Enzyme activity has previously been reported as an indicator of microbial activity or other soil biological properties, but this correlation is not always correct and can lead to confusing discussions around enzyme activity and microbial activity (Nannipieri et al., 2018). This makes studying enzyme activity important in seeing their functions in a soil environment. Soil organic matter is often synthesized and degraded by microbial enzyme activity, as well as contributing to many enzymatic properties that determine soil fertility and plant productivity (Burns et al., 2013). In addition, microbial extracellular enzymes that play a role in nutrient cycling can remain in the soil system even after microorganisms decay, while also helping in determining the effects that elevated atmospheric carbon dioxide and frequent wetting and drying cycles have on enzymatic activity (Burns et al., 2013). It has previously been noted that individual soil enzyme measurements can reveal specific decomposition processes and nutrient cycling that occur in soil (Nannipieri et al., 2018). From the study of enzymatic activity, it was discovered that irradiation of soil at the right intensity could yield highly, measurable urease and phosphatase activities (Nannipieri et al., 2018). In previous research, it was found that the use of p-nitrophenyl phosphate as a substrate can yield rapid and precise assay of soil phosphatase activity and can be done through colorimetric estimation of the p-nitrophenol released after incubation (Tabatabai & Bremner, 1969). Urease activity is often used to represent organic nitrogen mineralization, giving insight into organic nitrogen and nutrient cycling in the soil (Nannipieri et al., 2018). Both urease and phosphatase, being extracellular enzymes, are produced as a means to obtain organically bound phosphate and nitrogen (Van Der Heijden et al., 2008). Soil-bound enzymes may be indicative of substrate turnover during periods when microbial biomass is low or shut down due to stressed conditions, with the fact that much total activity of few extracellular enzymes is associated with organic and inorganic colloids (Burns et al., 2013).

Soil respiration is another viable tool in determining the decomposition of soil organic matter (SOM) through the evolution of carbon dioxide (CO2) (Ryan & Law, 2005). It has previously been noted that CO2 emissions have accelerated in the presence of nitrogen-based additions to agricultural soil, making the use of soil respiration relevant in turfgrass soils which are commonly supplemented with urea fertilizers (Azeem et al., 2020). Changes in the belowground carbon pool can be reflected in the carbon storage in terrestrial ecosystems and carbon flux to the atmosphere, making the CO2 efflux from the SOM surface indicative of belowground activity and microbial respiration (Ryan & Law, 2005). This link can be attributed to root activity and the activity of microorganisms in the rhizosphere being combined when discussed in the discussion of soil respiration (Hanson et al., 2000). Soil respiration shows great potential as an indicator of ecosystem metabolism, even having the ability to link soil respiration with aboveground processes like photosynthesis (Ryan & Law, 2005). With SOM being affected by microbial populations and their activity, by studying soil respiration in combination with SOM there can be an insight into the flow of energy and nutrients in a soil system and soil health (Barrios, 2007; Hanson et al., 2000; Ryan & Law, 2005; Van Der Heijden et al., 2008)

Microbial community composition, soil respiration, and enzyme activity have all been used in past studies as soil health indicators for turfgrass systems. However, there is a gap in knowledge between soil microbiology and golf course management, making research on the topic important. A previous study looking at the toxic effects of nano-pesticides on microorganisms, used quantitative polymerase chain reaction (qPCR) and urease activity in determining the microbial activity of the soil, determining there was a significant reduction of microbes after application (Liu et al., 2014). In addition, a study using Kentucky bluegrass turf receiving the pesticides bandane and calcium arsenate studied enzymatic activities and microbial abundance in determining the effects these chemicals have on soil health (Cole & Turgeon, 1978). In a study on the age of turfgrass systems, it was found that soil microbial biomass, activity, and nitrogen transformations are affected by the age of the turfgrass and management practices, some challenges being soil compaction and abrupt changes in landscape cover, changes in plant residues going back into the turfgrass system, and even foreign sand and subsoil used to replace surface soil (Shi et al., 2006). This is because, as a turfgrass system ages, the soil microbes will progressively be challenged by the changing environments associated with longer-term management practices instituted by superintendents (Shi et al., 2006). It was suggested that in younger turfgrass systems, soil microbes obtained their energy, carbon, and nitrogen primarily from fresh organic matter, but that as the turfgrass system ages, at a 0-5cm depth, the biomass carbon and nitrogen were significantly higher than younger systems (Shi et al., 2006). In a study examining soil microbial dynamics and carbon accumulation in highly managed turfgrass systems, it is noted that the conversion of agricultural cropping land to turfgrass results in increased microbial activity in that system (Wang et al., 2014). Using the incubation-alkaline absorption method, soil respiration was measured as a method of microbial activity, finding that in an 80-year highly managed turfgrass system, r-strategists microbial species became more dominant because of their ability to quickly adapt to frequently disturbed environments (Wang et al., 2014). The article included that reducing anthropogenic management intensities may facilitate the development of microbial communities and even foster carbon retention in turfgrass soils (Wang et al., 2014).

Oxygenated nanobubble water and its potential applications

One such technology is nanobubble technology and its effects on agriculture and ornamental production. This technology is based on the production of nanobubbles and their applications to provide benefits to soil and plant production. Nanobubbles are small in size, being below 1000nm in diameter, and are pockets of gas-filled cavities that can have a large surface area per unit volume (Atkinson et al., 2019). As a result of their size, they can have a concentration that may reach as high as hundred million to ten trillion bubbles per milliliter of liquid (Atkinson et al., 2019). Nanobubbles are generally negatively charged in a pH range that is common in the environment (2 to 12) (Temesgen, 2017). A significant quality of nanobubbles is that they can remain stable in liquid for an extended period, even being present for up to several weeks (Atkinson et al., 2019). Nanobubbles have many techniques for generation, including sonication, electrolysis, cavity formation, and the use of membranes (Phan, 2020). Focusing on the methodologies, the membrane method uses a nanosized membrane to push gas of a certain size into a flowing liquid, joining the nanobubbles with the liquid (Phan, 2020). May gases have been used in the production of nanobubbles, the most notable of which is compressed oxygen. This is because the infusion of oxygenated nanobubbles into irrigation water can have a multitude of benefits, one of which being the development of reactive oxygen species (ROS) that may rupture/collapse and act as a pathogen suppressant (Atkinson et al., 2019).

As a result of heavy rains, poor drainage techniques, or over-irrigation, problems with oxygen deficiency in the soil can occur. For instance, oxygen diffusion rates decrease in soil following irrigation or after rainfall, often decreasing even more in turfgrass soils that show compaction (Roberts et al., 1992). Without the necessary oxygen, functions in the root system cannot occur. In turfgrass, submerged roots cannot establish an osmotic gradient which can cause a net water loss due to restrictions on water transport to the shoot (Kopp & Jiang, 2015). Plant physiology is further affected, with plants becoming oxygen deficient due to the slower transfer of dissolved oxygen into water-filled pore spaces of the soil (Drew, 1997). Using the application of oxygenated nanobubble water, oxygen will be able to reach these oversaturated and compacted zones in the soil, resolving rootzone oxygen deficiency and leading to improved root performance, water use efficiency, and biomass (Lei et al., 2016).

In focusing more on the effects of compaction and oversaturation on root physiology and development, low oxygen is a primary root stressor, with this stress occurring predominately in saturated or flooded soils (Stier et al., 2015). The availability of oxygen in soil influences root properties. Root growth and maintenance are critical for plant survival when exposed to reduced oxygen levels and higher temperatures, as the temperature optimum for roots is less adaptable to these temperature extremes resulting in lower viability of the turfgrass under these heat stressors (Huang et al., 1998a). This can also be true about drought conditions from temperature extremes that may stress the field and soil, causing lower viability of the roots. Roots can follow moisture deep into the layers of the soil profile, with the ability of the turfgrass plant to avoid short-term or long-term drought (Huang, 2008). However, this can be problematic in the long run due to roots growing deeper into the soil leading to the rapid depletion of water in the plant (Huang, 2008). Root hair growth is also known to increase in length in response to nutrient diffusion decreasing in drier soils. This may be an adaptive mechanism to increase surface area and provide a greater root surface with more nutrient adsorption (Huang, 2008). This can be problematic because as roots continue to diffuse deeper into the soil layers, there will be even more oxygen restriction. Due to soil oxygen incorporation into traditional farmland relying on air diffusion, there is a limit of oxygen in soils and especially in deep soil layers (Wu et al., 2019). Higher temperatures and low oxygen levels in the rooting zone can negatively affect root development. The incorporation of oxygen with oxygenated nanobubble water may improve the oxygenation of soils in times of deficiency in the rooting zone. It was found that in oxygenated treatment zones, there was increased root access to oxygen as well as roots exhibiting higher respiration, with the use of aerated irrigation water for cotton production, and was shown to increase the soil oxygen level by two-fold (Pendergast, 2014). There is not only an effect of low oxygen on root properties but on the soil microbial communities residing within these rhizosphere zones.

Soil aeration is essential to the survival of soil microbes, with oxygen deficiency attributed to poorer soil health. When soil is warm and respiration is high by soil microorganisms, this reduction of oxygen can occur in less than 24 hours, transitioning the root environment from aerobic to anaerobic (Drew, 1997). It’s been researched that oxygen deficiency in turfgrass soil can cause the build-up of anaerobic metabolism products such as ethylene, carbon dioxide, and ethanol, all of being harmful to plant growth (Isweiri et al., 2022). These stressors will inhibit the production of quality turfgrass. Soil microorganisms have an important function in the cycling of nitrogen, sulfur, and phosphorous, as well as the decomposition of organic residues (Nielsen et al., 2002). Because of their essential part in soil health, changes in microorganisms respond quickly to changes and rapidly adapt to the environmental conditions to which they are exposed. As a result of these organisms being sensitive to anthropogenic disturbance, they make excellent indicators of soil health (Nielsen et al., 2002).

Nanobubbles might stimulate microbial activity due to improved aeration and improve many of these issues discussed. This is due to soil microbial respiration and the infusion of nanobubbles with oxygen providing more oxygen to these subsurface microorganisms. With the application of oxygenated nanobubble water, this technology stimulated soil respiration and microbial proliferation through improved soil aeration conditions, positively affecting microbial activities (Zhu et al., 2019). In addition, the oxygenated nanobubbles provide crucial oxygen to improve bio-decomposition by soil microbes (Wu et al., 2019). Increased aeration can stimulate microorganisms that are critical in the decomposition of organic matter that can attribute to the thatch layer and mineralization of nutrients to be used by the turfgrass. It is also possible that nanobubbles might negatively impact them because of the production of ROS. As such, research needs to be done to understand how oxygenated nanobubbles affect the soil microbial communities present within turfgrass, as soil microbes play a significant role in the establishment and maintenance of turfgrass systems. The process of decomposition is an important role that microorganisms play, which is usually controlled by heterotrophic microorganisms that lead to the cycling of nutrients like nitrogen (N), sulfur (S), and phosphorous (P), and the immobilization of carbon (C) and other nutrients (Murphy et al.). These nutrients, being released from their organic forms into inorganic forms, can then be used by the turf to prevent excessive accumulation of the thatch layer. One of these nutrients is nitrogen, which in its inorganic form can increase decomposition in soils (Parr & Papendick, 2015). It is cited that processes mediated by microbial species rely heavily on the functional diversity of the microbial population, including the range and complexity of carbon-substrates that a population can decompose (Murphy et al.). Plant-microbe interactions in the rhizosphere influence crop yield significantly, as the region around the root is relatively rich in nutrients because up to 40% of plant photosynthates are lost from the root system (Maheshwari et al., 2012). As a result, the rhizosphere supports large microbial populations that can influence plant growth.

Low oxygen is harmful to turfgrasses because these ornamentals have a greater demand for oxygen as the temperature increases. As the temperature increases in mid-summer, the combination of these increased temperatures and poor soil aeration would prove more detrimental to the turfgrass than any other stressor (Huang et al., 1998b). In a study done testing high temperatures and poor soil aeration on bentgrass cultivars, it was found that there were significant declines in turf quality in soils with poor aeration when compared to the control group (Huang et al., 1998a). Not only was there a decrease in the turf quality from this oxygen deprivation combined with high temperatures, but the chlorophyll content, photosynthetic rate, and respiration also all declined (Huang et al., 1998b). With the incorporation of oxygenated nanobubble water, there can be an alleviation of this pressure from compaction and low oxygen in the soil, leading to better turfgrass physiology. With oxygen gas being responsible for more than 200 different reactions in plant cells, oxygenation can be used to alleviate hypoxia and anoxia in the rhizosphere (Baram, 2022). Moreover, in a study with oxygenated brackish water, there was a significant promotion effect on seed germination and seedling growth with a higher germination rate, germination potential, germination index, vigor index, and plant height (Zhu et al., 2021). There is a need for research into how oxygenated nanobubble water interacts with soil aeration, leading to changes in turfgrass quality, root quality, and soil microbial abundance and activity.

Lastly, nanobubble water can potentially have an impact on water movement and retention in the soil because of its effect on surface tension. In previous reports, nanobubbles have acted as bridging agents by bringing together hydrophobic surfaces and influencing surface forces (Alheshibri et al., 2016). With the potential in reducing repellency due to hydrophobic surfaces in turfgrass soils, nanobubbles can improve water availability and reduce the need for chemicals used to fight localized dry spots, like wetting agents. There are also reports that oxygenated nanobubble water has the potential for improved water use rates in plants, which can potentially influence irrigation practices with nanobubble water incorporation (Yaxin Liu et al., 2019).

Research into nanobubble technology is still in its infancy, resulting in the study of oxygenated nanobubble technology in a turfgrass system being very limited as well. Linking how oxygenated nanobubble water affects golf course management strategies is of great interest, as the reduction of cost, resources, and quality pressures can be reduced as well. This research is necessary to find the effectiveness of oxygenated nanobubble water and push more sustainable production of turfgrass in the ever-urbanizing world.

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CHAPTER 3

EVALUATING THE EFFECTS OF OXYGENATED NANOBUBBLE WATER ON TURFGRASS GROWTH AND SOIL HEALTH IN A GREENHOUSE STUDY[1]

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ABSTRACT

The use of oxygenated nanobubble water in turfgrass has the potential to benefit the system by improving aeration. However, there is no research in this area. The objective was to evaluate the effects of oxygenated nanobubble water on turf growth and quality, water retention and movement, and soil biological health. A greenhouse trial was conducted with two treatments (nanobubble vs regular water) at two moisture levels (50% or 100% evapotranspiration) for three years. The 2021 and 2022 trials focused on evaluating impacts on turf growth and water retention and infiltration, while the 2023 trial focused on impacts on activity of soil microbial communities. Turf growth and quality showed varying results on some response parameters. For instance, clipping weight in 2021 showed significant treatment effect, with nanobubble treatment yielding a higher mean weight than the control, but in 2022 the opposite was true. In turf quality, visual quality score showed significant treatment effect in 2021, with the nanobubble treatment yielding a higher score than the control, but the same result was not seen again in 2022. For water movement, oxygenated nanobubble treatment impaired infiltration, with the rate being slower in the treatment than control. There were no significant treatment effects of oxygenated nanobubble water on soil biological health parameters (soil respiration and enzyme activities). Overall, oxygenated nanobubble water did not impact turf or soil health parameters probably due to the short residence time of the bubbles in soil or oxygen not being a limiting factor in the rhizosphere.

INTRODUCTION

The maintenance of golf courses involves a substantial input of resources, including fertilizers, water, and pesticides, making the golf industry a costly and resource-heavy sector of agriculture. It has been suggested that with a total turfgrass area of 163,812 km^2 in the United States (U.S), turfgrass represents the single largest irrigation crop in the U.S with an area three times larger than irrigated corn (Johnson et al., 2015). Research continues to identify the issues and benefits associated with the use and maintenance of turfgrasses, but within the past 20 years, the focus has changed from solely improving its aesthetic quality to also reducing the environmental impact of turf management as well (Johnson et al., 2015).

The shift in attitudes has led to the interest of superintendents in the use of products that are considered sustainable and environmentally friendly to their counterparts. One of these products is nanobubble technology which produces oxygenated nanobubbles in water. The use of oxygenated nanobubbles water has been gaining popularity within the past few decades for its potential to improve crop and ornamental productivity (Kalogerakis et al., 2021). The use of oxygenated nanobubbles in the turfgrass industry is still in its infancy. It has the potential to improve turfgrass growth and quality and sol biological health because of the impact it might have on soil aeration. Such benefits can translate into the use of less input in maintaining turfgrass, resulting in both financial and environmental benefits.

When soil experiences oxygen stress, it can inhibit root respiration, nutrient uptake, plant growth, leaf expansion, and photosynthesis rate, and overall, negatively affects yield (Baram, 2022). Moreover, Bermudagrass (*Cynodon dactylon*), being a warm-season grass used for golf courses in warm climatic regions, grows most rapidly in the summer months and can require frequent irrigation to maintain high-quality grass (Lu et al., 2008). Drought stress is especially important in arid climates as well, as 90% of water resources are generally allocated for use in agriculture (Soltani-Gerdefaramarzi et al., 2022). Due to limited water resources and increased energy costs, there is an increasing demand to lower irrigation requirements.

Nanobubbles have many unique characteristics such as extremely high surface area/volume ratios, enhanced solubility of oxygen in water, generation of free radicals, and their ability to persist suspended in a liquid for an extended period (English, 2022). It has been reported that the use of oxygenated nanobubble water on clayey soil that had been previously degraded by long-term irrigation of wastewater resulted in improved oxygenation in the root zone, as well as a decrease in nitrous oxide emissions (Baram, 2022). In addition, it has been reported that under an appropriate dissolved oxygen concentration, irrigation with oxygenated nanobubble water increased maize growth, yield, and nutritional quality (Zhou et al., 2019). Moreover, oxygenated nanobubble water has been reported to improve irrigation water use efficiency while increasing yield and fruit quality in tomatoes and cucumbers and hence has the potential to improve the sustainability of the turfgrass system (Liu et al., 2019).

With the potential benefits to the turfgrass system, it is important to evaluate the impacts of oxygenated nanobubble water on turfgrass growth and quality, water retention, and movement as well as on soil microbial communities that might potentially be affected due to the infusion of oxygen in nanobubbles into the root system. The objective of this study was, therefore, to determine the impact of oxygenated nanobubble water on turf growth and quality, water retention and movement as well as soil biological health in greenhouse studies.

MATERIALS AND METHODS

Turfgrass cultivar & transplanting

TifEagle bermudagrass turfgrass plugs were taken from a mature TifEagle green on the UGA Griffin campus (33.260457, -84.280543). The greenhouse experiment was repeated 3 times and plugs were taken in January from the field. These plugs were 10cm in diameter and 15cm in length. The bottom of the plugs was cut horizontally to an equal and flat length and leaving about two centimeters in thickness below the thatch layer. These plugs were then transplanted into pots that were 10cm in diameter and 38cm in length. There was a plastic mesh cloth located at the bottom of each pot. The pots were filled with a calcined clay mix to approximately 31cm to the top, leaving about 7cm from the lip of the pot. The plugs were then placed on top of this S&C mix and placed where the bottom of the plugs was touching the top of the S&C mix.

Turfgrass establishment

The pots underwent a period of establishment in the University of Georgia Griffin Campus in the Turfgrass Greenhouses (33.263017, -84.283657). During this period, the pots were watered 3x wk^{-1} with tap water from the hose, as well as fertilized with MiracleGro water-soluble all-purpose plant food (24-8-16) every other week. This establishment period spanned approximately 2 weeks in each of the three studies The greenhouse uses a VeriSTEP system for the establishment period and for the trial, which was set to 24℃ +/- 5.5, with a relative humidity of 70%. The system would turn on the lights at 7 am and off at 7 pm, with the lights turned on below 400 μM and on above 500 μM over the period of the day. General Electric GE Lucalox Lamps (1000 Watts) were used through the establishment period and the treatment trial period.

Experimental set-up

The 2021 and 2022 trials focused on plant and water factors, while the 2023 trial was performed to evaluate the impact of the technology on soil health. In the greenhouse, 20 pots were organized in 4 rows, with each row having 5 plants. The pots were evenly spread on each bench, with a random block design. There were two treatments of oxygenated nanobubble water and control. Both control and treatment used deionized tap water. There were 10 pots for each treatment and 5 replicates. Each treatment included two sub-treatments to vary the soil water level. The sub-treatments were 50% evapotranspiration or 100% evapotranspiration. As a result, we produced 5 drought-stressed pots replenished to 50% ET and 5 field capacity pots replenished to 100% ET within each treatment. This results in four combinations, each with 5 replication: 1.) control group irrigated to replace 50% ET 2.) control group irrigated to replace 100% ET 3.) oxygenated nanobubble water treatment irrigated to replace 50% ET, and 4.) oxygenated nanobubble water irrigated to replace 100% ET. (5 * 4 = 20). The experimental design visual aid is shown in Figure 3.1 but is not representative of how the pots were set up in the greenhouse.

After the establishment period, irrigation treatments continue to be applied 3x wk^{-1}, but solely replacing the 50% ET and 100% ET with the exact amount of water needed. The application schedule for 2021 is shown in (Fig. 3.2) and the application schedule for 2022 is shown in (Fig. 3.3). The application schedule for the 2023 trial is shown in (Fig. 3.4).

Yielding the exact 50% ET and 100% ET irrigation water needed to replenish the pots for the sub-treatment has a unique procedure. Pots were watered to saturation and allowed to drain overnight to reach field capacity. For the irrigation treatments, pots were weighed, and 100% ET treatments received enough water to bring pots back to field capacity. For the 50% ET treatment, the actual 100% ET yielded was halved and this amount was used to bring the remaining sub-treatment pots back to 50% ET.

In the production of nanobubble water for the greenhouse trials in the spring of 2021, the nanobubbler was run for 1 hour at an approximate volume of 13-15 liters using a Japanese Anzai Moleaer unit (Moleaer, Carson, CA) unit and a compressed oxygen tank. The total dissolved oxygen (DO, mg L^{-1}) was taken using a dissolved oxygen meter (HI98193, Hannah Instruments) after the 1-hour mark with the nanobubbler turned off and was measured before it was applied to the nanobubble pots. The average dissolved oxygen level over the term of this study was 37.29 mg/L. The production and stability of the dissolved oxygen in the oxygenated nanobubble water for this nanobubbler were recorded (Fig. 3.5). In the production of nanobubble water for the greenhouse trial in the spring of 2022 and 2023, the nanobubbler was run for 30 minutes at an approximate volume of 15 liters using a Rapid Waters Technologies (Grand Rapids, MI) nanobubbler and compressed oxygen tank. The total dissolved oxygen (DO, mg L^{-1}) was taken after the 30-minute mark with the nanobubbler turned off and was measured before it was applied to the nanobubble pots. The average dissolved oxygen level was 48.12 mg/L. The production and stability of the dissolved oxygen concentration in the oxygenated nanobubble water for this nanobubbler were recorded over 10 minutes (Fig. 3.6) and over 6 hours (Fig. 3.7). Table 3.1 shows the loss in dissolved oxygen when oxygenated nanobubble water is removed from the tank and applied to treatment pots.

The 2023 soil health trial lasted from January 31st to March 3rd, 2023. After the trial period, the pots were dismantled on March 3rd, 2023. Soil samples were removed from the rhizosphere and placed into 1 gallon-sized Ziploc bag. Soil samples were sieved through a 2mm sieve before processing for soil moisture and soil biological health as described below.

Measurement of plant growth parameters

To evaluate the response of the turfgrass to oxygenated nanobubble water, the following parameters were measured twice per week: clipping weight, relative leaf water index, turf quality, pot weight, and infiltration rate. The following parameters were measured at the end of the trial: maximum root length, root weight, thatch layer weight, above-ground shoot weight, and root trait analysis. The measurement schedule for 2021 is shown in Fig. 3.8. The measurement schedule for 2022 is shown in (Fig. 3.9). For the 2023 soil health trial, turf quality was evaluated on February 2nd, February 15th, and March 3rd, but no other turf growth parameters were taken during the duration of that study (Fig. 3.4).

Clipping weight

Clipping weight was measured using a pair of scissors (Westcott, Titanium) and cutting the grass flush with the top of the pot. These clipping samples were placed into #1 coin envelopes (Quality Park, 2.25in X 3.5in) and moved into a VWR gravity convection incubator at 70°C for 4 days or more. The dry clipping weight material is then removed from the incubator and weighed to yield a dry clipping weight of the grass. A (Mettler AE 100) balance was used to take weight measurements for all trials.

Maximum root length

Maximum root lengths were acquired at the end of the study for all pots. Using a ruler, the length of the roots from the bottom of the thatch layer to the end of the longest root was taken and recorded. The roots were placed in a #1coin envelopes (Quality Park, 2.25in X 3.5in) and set in an incubator at 70°C for 4 days or more. A dry weight was then taken.

Thatch layer weight & Shoot Weight

Thatch layer weight was taken at the end of the study on all pots. This was done by using scissors and removing the grass located at the top of the thatch layer and removing the roots at the bottom of the thatch layer. The thatch layer was placed in a brown paper bag and set in an incubator at 70°C for 4 days or more. After this period, sand was shaken from the thatch layer, and the thatch layer was weighed.

Shoot weight was taken at the end of the study on all pots. Scissors were used to cut the grass removing all green tissues. This was to remove the thatch layer from the grass blades. The grass was placed into #1 coin envelopes (Quality Park, 2.25in X 3.5in) and set in an incubator at 70°C for 4 days or more. After this drying, the dry weight of the grass samples was measured.

Root scan analysis & root weight

Roots were removed from the pots, washed, and ready to be scanned. The roots were laid out on a plastic tray. An Epson scanner (Epson Perfection V550 Photo) was used to scan the roots. The plastic tray was 30.48 cm in length, 21.59 cm in width, and 1.27cm in height to fit the scanner, as well as be able to sustain a thin layer of water. This thin layer of water was used to suspend the representative roots in an aqueous solution. The roots were placed in this DI water bath fully covering the plastic tray. Debris and organic matter were removed from the root samples. The tray and root samples were scanned, producing a high-quality JPG image that was saved to the computer. These images were run through GIAroots digital imaging root analysis program (Galkovskyi et al., 2012) to yield the average root width, the maximum number of roots, the median number of roots, and number of connected components.

After the scanning process, the root samples were removed from the plastic scanning tray and placed into #1 coin envelopes (Quality Park, 2.25in X 3.5in) and into an incubator at 70°C for 4 days or more. After this period the root samples were removed from their envelopes and a root dry weight was recorded.

Turf quality with digital imaging & visual rating

For digital imagery, a Canon G9X digital camera with the custom settings of Iso: 400, flower interface, F: 4.0, and custom white balance of 0 was used. A PVC Lightbox was used to capture an image of the canopy to ensure uniformity in lighting. One image was taken per pot. The images were run through Fiji ImageJ software (Schindelin et al., 2012) to calculate the percent green cover providing an objective assessment of the overall turf quality and quantitative data for future statistical analysis. From this, a percentage of the green cover area of greenness was determined (Jespersen et al., 2019). A visual rating was also performed on each pot to assess turfgrass quality. It was based on a scale of 1-9, using color, density, and uniformity to determine the rating (Krans & Morris, 2007).

Measurement of water movement and retention

Leaf relative water content

Leaf relative water content (RWC) was taken through cuttings from the pots. After cuttings were taken, they were wrapped in aluminum foil and iced to be brought to the lab. There, a fresh weight (FW) was taken of the clippings. These leaf tissues were then brought to a turgid weight (TW). This was conducted through the clippings being submerged in DI water overnight in a refrigerator at 2.8℃ and being reweighed the next morning. After being weighed, the clippings were placed into #1 coin envelopes (Quality Park, 2.25in X 3.5in) and moved into a VWR gravity convection incubator at 70°C for 4 days or more. Dry weight (DW) was taken from these clippings using a (Mettler AE 100) balance after the allotted time (Katuwal et al., 2020). The equation used to calculate was:

$$RWC = [(TW-FW) / (TW-DW)] * 100$$

Infiltration rate

The infiltration rate was determined by using a cylinder with a height of 10.6mm and a diameter of 6mm. This yields a total volume of 299.71mm^3. From this cylinder, there is a notch in the side, 6mm high with a volume of 170mm^3. Water, whether it be the treatment or control irrigation, was filled up to this line. The timer was started immediately when the water reaches this line and the timer was stopped when the water had fully infiltrated into the pot and no water was left within the cylinder. This calculation yielded an infiltration rate with the units of mm^3/sec.

Measurement of soil biological health indicators

Phosphatase assay

Soil sample extracts were colorimetrically analyzed in estimating the rate of phosphatase activity (µmol phosphate evolved $g^{-1} \cdot h^{-1}$), acting as an indicator of soil P cycling as described in Tabatabai & Bremner, 1969. Two brown 16-mL scintillation vials were used for each soil sample, ensuring the minimization of light exposure. One gram of the soil and 4 mL of Tris-maleate buffer were added to each vial. One milliliter of 100-mM of para-nitrophenyl phosphate (PNPP) was added to one of the two vials acting as a treatment, with the other vial being the control. The vials were shaken for 30 minutes on a rotary shaker at 175 reps per minute (rpm). Once complete, one milliliter of pNPP was added to the control vial. One mL of 0.5 $CaCl_2$ and 4 mL of 0.5-M NaOH were added to all the vials, terminating all activity. The tubes were centrifuged for 10 minutes at 10,000 rpm at 4℃. The absorbance of the supernatant from each vial was colorimetrically analyzed using a spectrophotometer at 400nm. Select samples were diluted to 1:10. Standard curves were produced, ranging from 0 to 30 µM p-nitrophenol in Tris-maleate buffer for each set of samples. From this, linear equations are developed and used to calculate phosphatase concentration in each vial (µmol phosphate L^{-1}), as one mole of phosphate is produced by one mole of p-nitrophenol. The difference between phosphate concentrations in the treatment and control vials was used in the equation below to determine phosphatase activity (µmol phosphate evolved $g^{-1} \cdot h\text{-}^{-1}$) in each soil sample:

$$\mu\text{mol } P_i \text{ evolved } g^{-1} \cdot h^{-1} = (\mu\text{mol } P_i/\text{ L}) \times (10 \text{ mL}/1 \text{ g}) \times (1 \text{ L}/ 1000 \text{ mL}) \times 2$$

Urease assay

In the urease assay, soil samples were analyzed using a 2% boric acid trap method to indicate the rate of urease activity (µmol NH_3 evolved g^{-1}) as an indicator of soil N cycling as described in Mobley and Hausinger (1989). For each sample, two bi-plate petri dishes were prepared by adding 1 g of soil and 3 mL of 0.5-M Tris-maleate buffer solution (pH 7.0) with 1% sodium azide in one section of the bi-plate. Three milliliters of the 2% boric acid indicator solution were pipetted into the second section of the bi-plate of each petri dish. 1 mL of 6-M urea was pipetted into one replicate of each soil sample, as it is used to initiate enzyme activity. Petri dishes were incubated for exactly one hour at room temperature. After this period, 0.5 mL of 10-mM $AgSO_4$ solution and 3-M K_2CO_3 solution were added to terminate urease activity and release evolved NH_3 into the boric acid trap. After the addition of these solutions, the plate was secured in Ziploc bags and was allowed to incubate for 24 hours at room temperature. The boric acid solutions were then titrated using a 0.02-*N* HCl solution. The rate of urease activity for each soil sample was calculated by applying the equation to the amount of HCl used to titrate each petri dish and subtracting the value of the control from the value of the treated plate:

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

Soil Respiration

To test the effect of treatments, soil respiration (mg CO_2 evolved g^{-1} soil*d^{-1}) was used as an indicator of microbial activity, as described in Zibilske (1994). Twenty grams of soil was used from each sample and placed in an individual mason jar. An empty jar was obtained to be used as a control and capture background CO_2. Glass beakers composed of 10 ml of 0.08-*N* $Ba(OH)_2$ were placed into each mason jar to capture the evolved CO_2. The mason jars were sealed with the alkaline trap inside and allowed to incubate at 30°C for 24 h. Hydrochloric acid (0.08-N) was used to titrate the $Ba(OH)_2$ traps in the presence of an indicator, phenolphthalein. The carbon dioxide from the control jar was subtracted from the jars with soil and the soil respiration was estimated with the equation below, x being the volume of HCl it took for titrating the trap.

$$\text{mg } CO_2 * g^{-1} \text{ soil} * d^{-1} = [0.08 \text{ N } Ba(OH)_2 * 10 \text{ mL } Ba(OH)_2]$$
$$-[0.08 \text{ N HCl} * (\text{HCl Control-HCl Treatment}) \text{ mL}] * [22/(\text{dry soil weight (g)})]$$

Soil moisture

To determine the soil moisture of the samples, 10 g of fresh soil was weighed (Model Adventurer Pro AV2102C, Ohaus Corp., Pine Brook, NJ) into the aluminum tin and dried for 24 hours in an oven at 100℃. The soil was cooled in a desiccator and weighed to obtain oven-dry weight and the gravimetric water content was determined as follows:

Gravimetric water content (g) =[(Fresh Weight-Dry Weight) / (Dry Weight-Tin Weight)] *100

Statistical analysis

Analysis of variance (ANOVA) was carried out in testing the statistical significance of the effects of oxygenated nanobubble water on turf quality and indicators of soil health at a significance of 0.05 in JMP Pro 16. The method used for the analysis of variance was repeated measures analysis as a split-plot ANOVA, to find the significance of oxygenated nanobubble treatment (T) and 50% ET and 100% ET sub-treatment (ST) on plant growth parameters, with replication considered a random effect. Tukey's honest significant difference (HSD) test was used to identify significant relationships among treatments within all models. Mixed model ANOVA tables and Tuckey's tests were distinguished by year, as there was a change in nanobubble technology to produce the oxygenated nanobubbles for use in treatment applications.

RESULTS AND DISCUSSION

Clipping weight

The clipping weight in the 2021 trial yielded significant sub-treatment*treatment interaction effects with the (100% ET)*Nanobubble interaction yielding a higher clipping weight of 0.043 g than the other interactions (Table 3.2 & 3.3). The (100% ET)*Nanobubble interaction was significantly different from any of the other interactions. The clipping weight in the 2022 trial yielded significant sub-treatment*treatment interaction effects with the (50% ET)*Control having a higher mean clipping weight of 0.077 g than the Nanobubble interactions with either of the 50% ET or 100% ET sub-treatments. There were no significant sampling date*treatment interactions for clipping weight for 2021 or 2022 (Table 3.2).

There is limited research done measuring turf growth through clipping weight during the alleviation of oxygen deficiency within the soil with the use of oxygenated nanobubble technology. In saying this, it has been noted that reduced soil oxygen availability decreases chlorophyll and carbohydrate concentrations, photosynthetic rate, and turf quality in a turfgrass system (Jiang & Wang, 2006). In a study on perennial ryegrasses that had been waterlogged for 28 days, the photosynthesis of all experimental entries of perennial ryegrass was reduced by 30-50%. There is a general decrease in turfgrass growth as soil oxygen is limited in the system. Because there were inconsistencies in the data from either trial, a lack of oxygen may not have been the limiting factor in these systems and may result in differences in clipping weights across years. There may have been an external factor that could have had more of an impact on clipping weight than soil oxygen.

Turf quality

The turf quality measured in the 2022 trial through digital imaging analysis for % area greenness showed significant treatment effects with the control yielding a higher mean % area greenness of 86.3% compared to that of the oxygenated nanobubble treatment with 85.4% (Table 3.3). This significance was not seen in the 2021 trial measuring % area greenness (Table 3.2). There were no significant sampling date*treatment interactions for turf quality through digital imaging analysis for 2021 or 2022 (Table 3.2).

The turf quality measured in the 2021 trial through visual scoring on a 1-9 scale showed significant sub-treatment*treatment interaction effects (Table 3.2). It can be seen in Table 3.3 that the sub-treatment of 100% ET and the interactions with nanobubble and control yielded higher means in visual scoring of 6.42 and 6.12 than the (50% ET)*Nanobubble visual score (5.95) and (50% ET)*Control visual score (5.42). There were no significant sampling date*treatment interaction effects for turf quality through visual scoring for 2022, but there were for 2021 (Table 3.2). For further data on the significance of sampling date*treatment interaction, Figure A.1 gives insight into the relation of treatments over time.

Many extraneous factors can affect turf greenness and visual scoring. For instance, mowing height, removal of grass clippings from a site, and the accessibility the turfgrass has to N, all play a role in turf quality and improved turf color in a golf course system (Kopp & Guilliard, 2002). Moreover, the percent area greenness and visual scoring, when showing the significance of treatment, were respectively close to each other for overall turf quality. For the percent green area for 2022, the treatment application showed to be significant, but the difference between the control (86.3% area greenness) and nanobubble treatment (85.3% area greenness) was not extensively far from each other. The same evaluation can be applied to the visual rating in 2021 showing the significance of treatment. The visual scoring for nanobubble treatment (6.42) and control (6.12) did not show to be extensively far from each other.

For the 2023 soil health trial, area percent greenness was taken at the beginning, at the middle, and at the end of the trial. There were no significant sub-treatment, treatment, of sub treatment-treatment interaction effects during the extent of the trial (Table 3.4). Looking at Table 3.5, the same can be said with there being a lack of significance even between sub-treatments of 50% ET and 100% ET. This lack of significance may have stemmed from the 2023 trial being time constrained. The difference in means between oxygenated nanobubble treatment and control did not show significant treatment effects, further supporting the use of the oxygenated nanobubble technology being up to the discretion of the turfgrass manager.

Root length, root weight, thatch layer weight, grass/shoot weight

There were significant treatment effects on maximum root length for the 2022 trial (Table 3.6). The maximum root length for the 2022 trial showed a higher mean maximum root length for the control (14.2 cm) when compared to the treatment (11.9 cm) (Table 3.6). There were no significant treatment effects on maximum root length for the 2021 trial. There were no significant sub-treatment or ST-T interaction effects for maximum root length for both trials.

There were no significant treatment or sub-treatment effects for root weight for both trials. There were significant ST-T interaction effects for the root weight in the 2021 trial (Table 3.6). However, there was a lack of significant treatment effect for the ST-T interactions represented in Tuckey's mean differences (Table 3.7).

There were significant treatment effects on thatch layer weight for the 2021 trial (Table 3.6). The thatch layer weight yielded a higher weight in the control of 39.0 g when compared to the nanobubble treatment of 30.1 g (Table 3.7). The result of significance in thatch layer weight was not replicated, as there were no significant treatment effects on thatch layer weight for the 2022 trial (Table 3.6). There were no significant sub-treatment or ST-T interaction effects on thatch layer weight for both the 2021 and 2022 trials (Tables 3.6 & 3.7).

There were no significant treatment or ST-T interaction effects on shoot weight for the 2021 and 2022 trials. There were significant sub-treatment effects for the 2022 trial (Table 3.6). The sub-treatment for 2022 shoot weight showed that 50% ET yielded a higher mean shoot weight compared to 100% ET. Referring to (Table 3.6), the shoot weight showed a higher mean in the (50% ET)*nanobubble interaction, and the lowest mean was in the (100% ET)* nanobubble treatment.

The results show some inconsistencies, which may be attributed to oxygen not being the limiting factor in this system. There may have been an external factor that could have had a more significant effect on these measured response parameters than oxygen deficiency, giving inconsistent data or the lack of a treatment effect.

Root scan analysis

There were significant treatment effects on average root width for the 2021 trial (Table 3.8). The average root width showed to be higher in the nanobubble treatment when compared to the control treatment (Table 3.9). There were no significant treatment effects on the average root width for the 2022 trial. There were no significant differences for sub-treatment or ST*T interaction effects on average root width for either the 2021 or 2022 trials. There were no significant treatment, sub-treatment, or ST*T interaction effects on the maximum number of roots, media number of roots, or number of connected components (Table 3.8 & 3.9).

The nanobubble treatment showed a higher mean than the control but was not significant. There was no significant difference for the remaining root parameters measured. There is limited research on the study of how oxygenated nanobubble water affects root growth. In a study using perennial ryegrass as a cultivar, it has been found that this turfgrass system was able to maintain high relative root growth even under oxygen-deficient conditions (Isweiri et al., 2022). If oxygen deficiency were a limiting factor in this experiment, the same results could have been for this greenhouse trial as well. There is also the potential that oxygen was not a limiting factor in this experiment and showed limited significance through these trials.

Relative leaf water index

The relative leaf water index yielded significant subtreatment*treatment interaction effects for the 2021 trial (Table 3.2). About Table 3.3, it can be seen that the (100% ET)*Control yielded a higher mean relative water content of 77.8% compared to that of the other interaction effects. The (100% ET)*Control interaction was significantly different than any of the other interaction affects. In the 2021 trial, there were also sampling date*treatment interaction effects, which can further be evaluated in Figure A.2, giving insight into the relation between sampling date and treatment. For the 2022 trial of the same measure, there were no significant subtreatment*treatment interaction effects or sampling date*treatment interaction effects, meaning that these significances were not replicated in this trial.

TifEagle bermudagrass is a warm-season grass that is bred to withstand hot summers, there is potential that varying relative water content could be indicative of drought tolerance. It has previously been noted that increased RWC under water deficit is associated with increased drought tolerance and that the intention of this measure is for measuring the plant's water status during water deficits (Kirigwi et al., 2022; Xu & Zhou, 2011). As a result, even though we had a sub-treatment of 50% ET during the trials, the irrigation deficit pots could have withstood drought tolerance better because of the use of warm-season grass. This may be a reason behind there being a lack of significant sub-treatment effect for the 2022 year, as even when Tifeagle bermudagrass is under irrigation stress, the RWC remains unaffected due to the turfs breeding to withstand drought treatments. This can also be seen in the there being significant sub-treatment effects in the 2021 sub-treatment means for leaf relative water content, as the difference in means was between 100% ET (74.8%) and 50% ET (72.4%) giving a 2.4% difference.

Infiltration rate

There were significant treatment effects on infiltration rate for both the 2021 and 2022 trials (Table 3.10). When comparing the mean infiltration rates, there is a faster infiltration rate in the control when compared to the nanobubble treatment (Table 3.11).

Phosphatase and urease enzyme activity

The control mean urease activity in 2023 was 7.75 µmol NH_3 g^{-1} soil h^{-1} and the treatment mean urease activity was 9.55 µmol NH_3 g^{-1} soil h^{-1} (Table 3.12). There were no significant treatment effects on soil urease enzyme activity (Tables 3.12, 3.13, & Fig. 3.10). The control mean phosphatase activity in 2023 was 0.243 µmol pNP g^{-1} soil h^{-1} and the treatment mean urease activity was 0.172 µmol pNP g^{-1} soil h^{-1} (Tables 3.12, 3.13, & Fig. 3.11). There were no significant treatment effects on soil phosphatase enzyme activity.

Urease activity can be an indicator of change potentially brought about by the oxygenated nanobubble technology on the nitrogen cycle. The absence of any significant impact indicates that the treatment with oxygenated nanobubble water did not influence the cycling of nitrogen. Phosphatase activity can be an indicator of change potentially brought about by the oxygenated nanobubble technology on the phosphorous cycle. The absence of any significant impact indicates that the treatment with oxygenated nanobubble water did not influence the cycling of phosphorus.

Both urease and phosphatase enzymes behave differently than other microorganisms as they are secreted in the rhizosphere and have the potential to exist in conditions that otherwise are intolerable by microorganisms. These enzymes are closely associated with the thatch layer in turf soils, resulting from protein molecules creating complexes with soil organic matter. Moreover, it has been suggested that most of the variation in urease activity among soils can be accounted for by the quantity of soil organic matter, with SOM protecting soil urease from microbial degradation without disturbing the enzyme activity (Torello & Wehner, 1983).

Because thatch is an extensive layer of soil organic matter that resides on the soil surface, there is potentially a high level of urease activity in the thatch layer. As a result, removing the thatch layer from the soil sample and isolating 6 inches below it to properly measure the microbial activity in the rhizosphere might remove some elements that contribute to these enzymatic activities along with the thatch layer (Mueller & Kussow, 2015). This can potentially affect the enzyme activity during laboratory analysis. In addition, it is possible that the limiting factor for urease and phosphatase activity was not oxygen stress. Another factor that can alter the enzyme activity could have been the longevity of the greenhouse system. Although turf plugs were established in pots, the time of the 2023 soil health study in the greenhouse was limited by time (<2 months). Microbial extracellular enzymes that play a role in nutrient cycling can remain in the soil system even after the death of microorganisms (Burns et al., 2013). The infancy of the turfgrass pots established in the greenhouse for the 2023 soil health trial may have been representative of a lack of enzyme development, as the microorganisms may not have been fully established in the pots when they were dismantled to perform in-lab soil health studies. The limiting factor of time may have caused there to be a lack of significant difference in treatment effect because the turfgrass system established was newer.

Soil respiration

The mean soil respiration for control in 2023 was 25.22 mg CO_2 g^{-1} soil d^{-1} and the mean treatment soil respiration was 26.51 mg CO_2 g^{-1} soil d^{-1} (Table. 3.12). There were no significant treatment effects on soil respiration (Tables 3.12, 3.13 & Fig. 3.12). One factor that could have accounted for the lack of absence is the short-term nature of the study. There may need to be a long-term study in the greenhouse to see any significant impact or that the system was not oxygen limited and that microbial activity was not responding to oxygenated nanobubble water.

Another limiting factor could have been the retention of dissolved oxygen in the application of oxygenated nanobubble water. Even when the oxygenated nanobubbler produced an average dissolved oxygen concentration of 48.15 mg L^{-1}, the concentration of dissolved oxygen when applied to the greenhouse pots through a beaker dropped to 24.53 mg L^{-1} (Table 3.1). In the future study of the effects of oxygenated nanobubble technology in a turfgrass system, it would be beneficial to add soil oxygen sensors to the greenhouse pots to fully investigate if the addition of oxygenated nanobubble water is reaching the soil within the pots. This is especially important, as the soil in the greenhouse was calcined, giving the clay a greater ability to retain water after irrigation than the regular 97% sand and 3% organic matter mix that is common in golf courses.

SUMMARY and CONCLUSIONS

Overall treatment effects showed varying results amongst all measurements of turfgrass growth and turfgrass quality. The clipping weight for the 2021 trial showed significant treatment effects with the nanobubble treatment yielding a higher mean clipping weight than that of the control. For the 2022 trial measuring clipping weight, the results were inverse, as the control yielded a higher mean clipping weight compared to that of the oxygenated nanobubble treatment.

Turf quality through digital imaging and percent area greenness showed significance for 2022, with control yielding a higher mean percent area greenness than the nanobubble treatment. This significance was not seen in the 2021 trial for turf quality. The turf quality through visual scoring showed significant treatment effects in 2021, but not in 2022, with the nanobubble treatment yielding a higher mean visual score compared to the control. For visual rating, there were significant ST*T interaction effects in 2021, as the 100% evapotranspiration and their interactions with nanobubble and treatment yielded a significantly different mean compared to that of any of the other interaction effects.

For root parameters, the average root width showed significance in 2021 with nanobubble treatment yielding ah higher mean average root width compared to the control, but this significance was not replicated in 2022. The same can be said for the median number of roots, as there were significant treatment effects with the nanobubble treatment yielding a higher mean of the median number of roots compared to the control, but this significance was not replicated in the 2022 trial. Maximum root length showed significance in the 2022 trial, with the control showing a higher mean of maximum root length compared to that of the nanobubble treatment, but this significance was not seen in the 2021 trial. Moreover, the thatch layer weight showed significance for the 2021 trial, as the control yielded a higher mean thatch weight compared to that of the nanobubble treatment, but this significance was not replicated in the 2022 trial.

There were also varying effects for the water retention, as the relative leaf water index showed significant treatment effects for the 2021 trial but did not for the 2022 trial. The relative leaf water index showed control yielding a higher mean relative leaf water index compared to that of the nanobubble treatment, but this significance was not replicated in the 2022 trial. There were consistent results found with infiltration rate measuring water movement, as the control yielded a higher mean infiltration rate than the oxygenated nanobubble treatment across both trials, which does not support the hypothesis. The use of oxygenated nanobubble water did not significantly impact any of the soil biological health parameters (enzyme assays and soil respiration) measured in the greenhouse trials.

There may have been external factors that were not considered through this study that had more of an effect on the turfgrass growth and quality, water movement and retention, as well as the soil microbial activity. In addition, there may have been a loss in dissolved oxygen when the oxygenated nanobubble water was applied to the pots, as there were losses in dissolved oxygen from the time of generation to the time of application. The retention of dissolved oxygen after application to the pots would require further study using in-situ sensors.

This study presents some insight into the dynamics between oxygenated nanobubble treatment and the response of turf growth, turf quality, soil microbial health and abundance, and turf physiology parameters in a greenhouse. The practical importance of the study is to provide information to golf course superintendents and turfgrass managers about new technologies that are marketed to them to enable them to make informed decisions.

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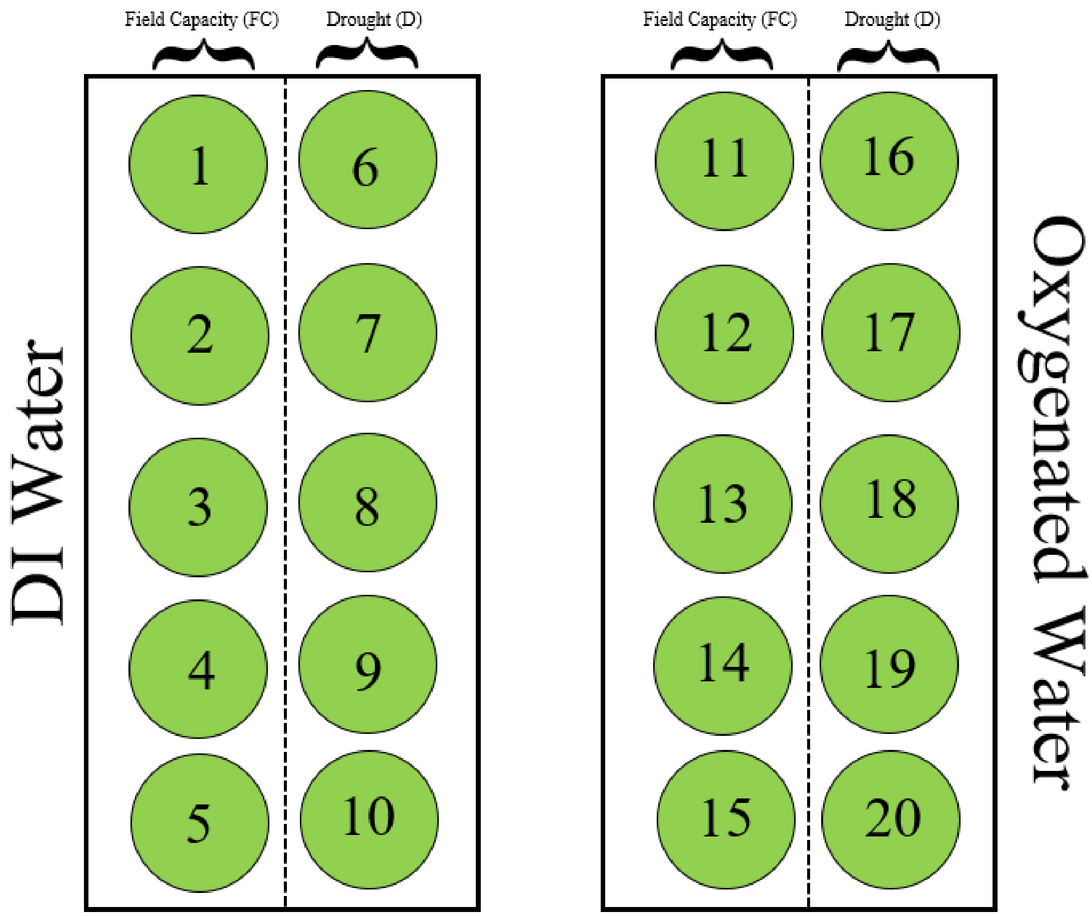

Figure 3.1: Experimental design for the greenhouse study. Twenty pots were organized in 4 rows, with each row having 5 plants. There were two treatments: oxygenated nanobubble water vs deionized water. Both control and treatment used deionized tap water. Each treatment had a sub-treatment of either 50% or 100% evapotranspiration to reflect two levels of water availability. This image is not representative of how the pots were set up in the greenhouse but is to show the treatments and their respective sub-treatments through visual aid.

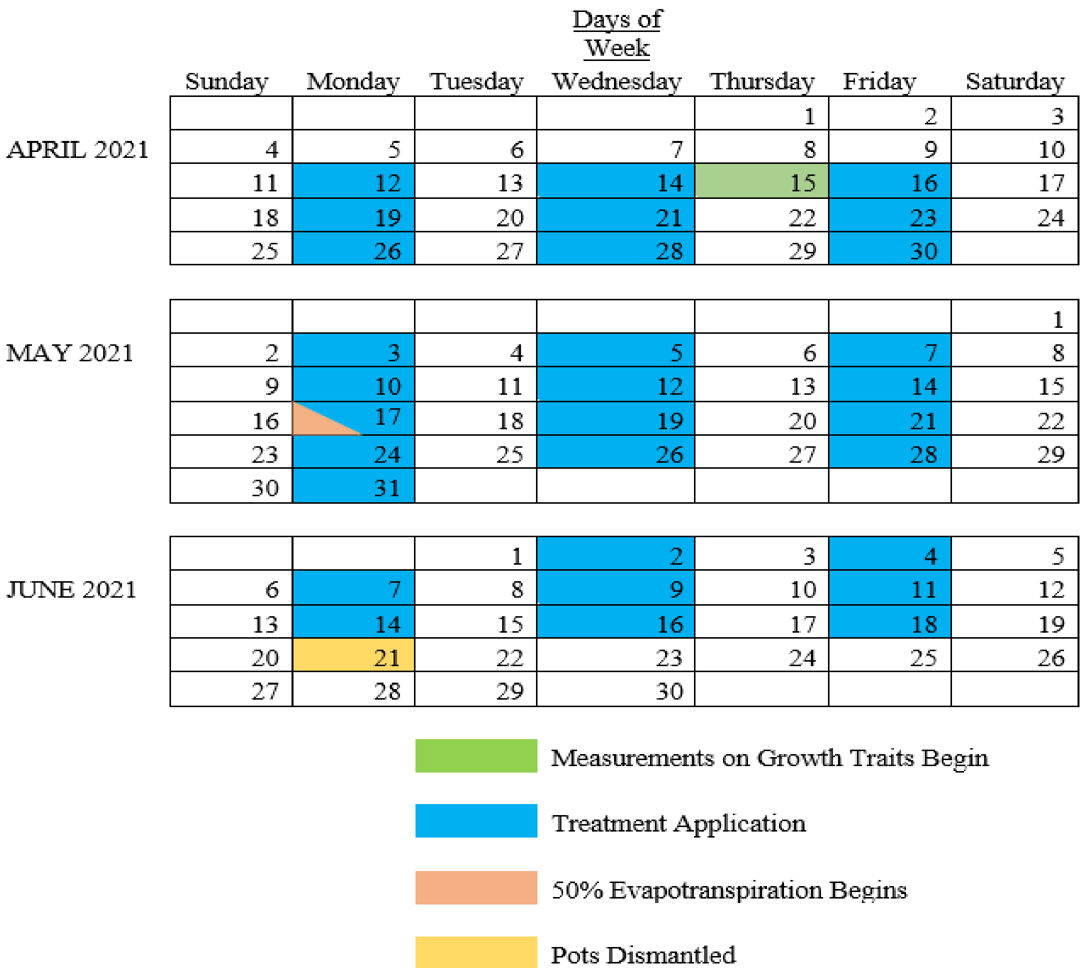

Figure 3.2: 2021 application schedule

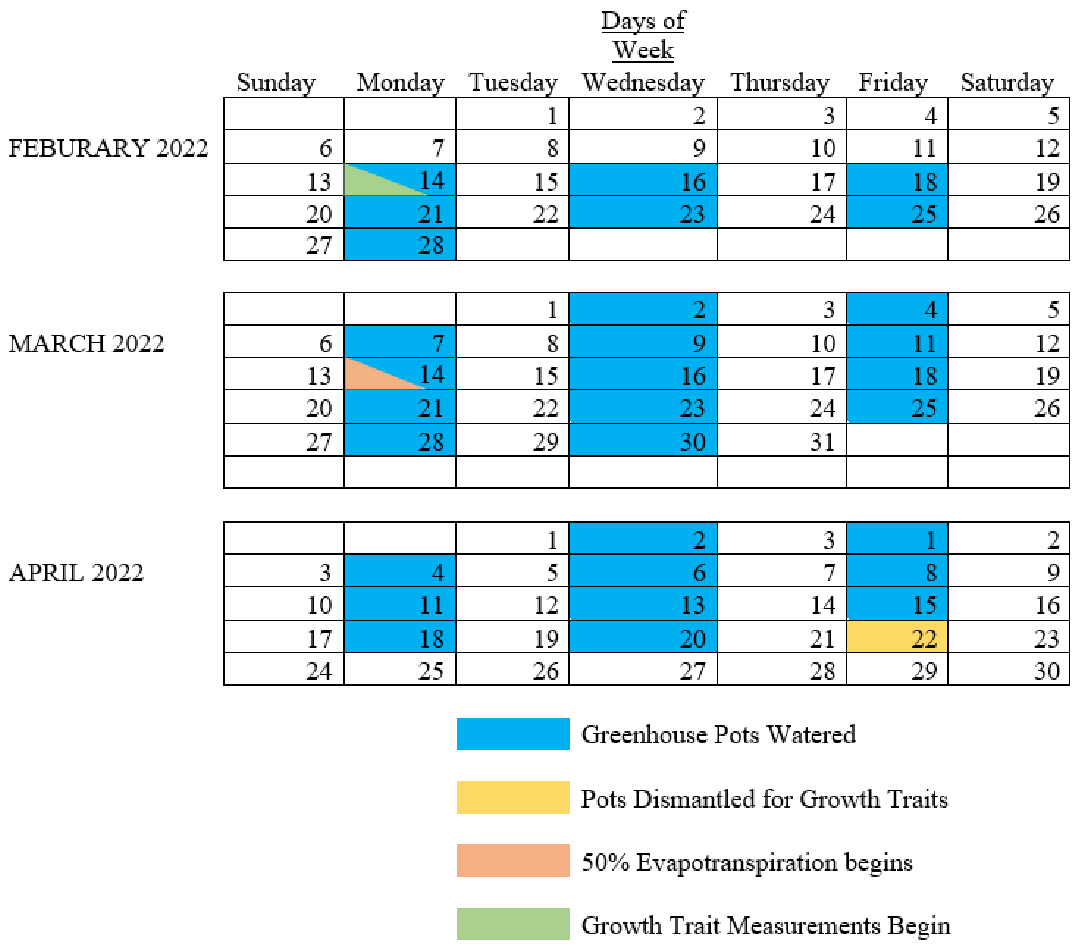

Figure 3.3: 2022 application schedule

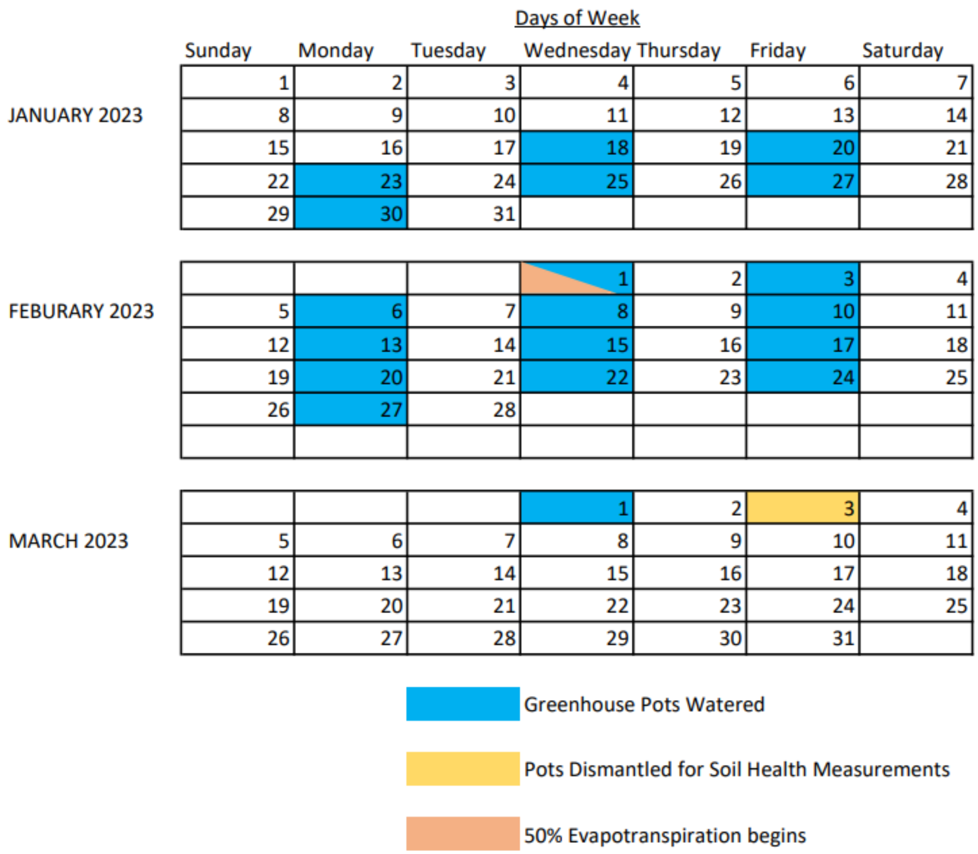

Figure 3.4: 2023 soil health study application schedule

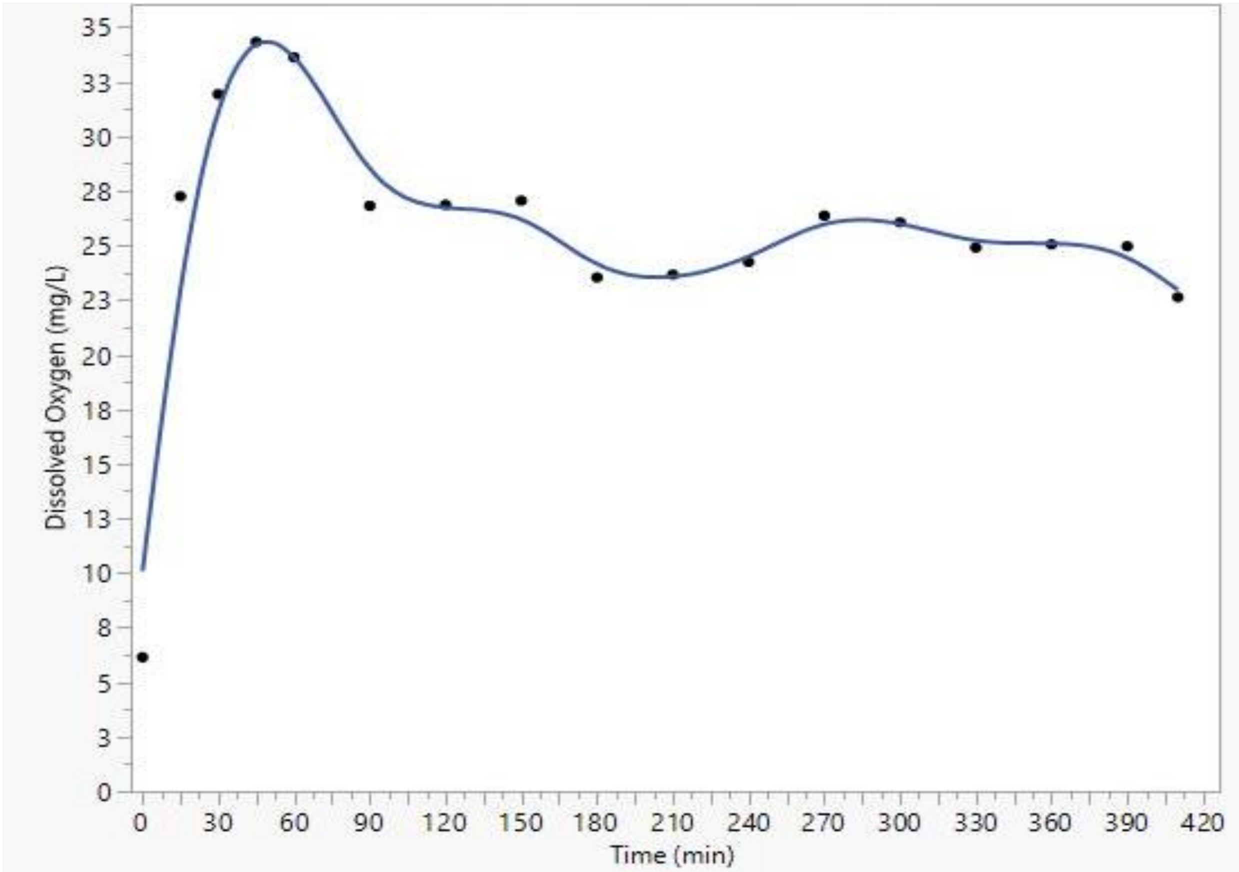

Figure 3.5: 2021 dissolved oxygen (mg/L) measurements to produce oxygenated nanobubble water using the Japanese Anzai Moleaer unit (Moleaer, Carson, CA) unit. The sixty-minute mark is the time the Japanese Anzai unit was turned off, with time afterward being the stability of nanobubbles suspended in deionized water. The dissolved oxygen concentration was measured at 15-minute intervals.

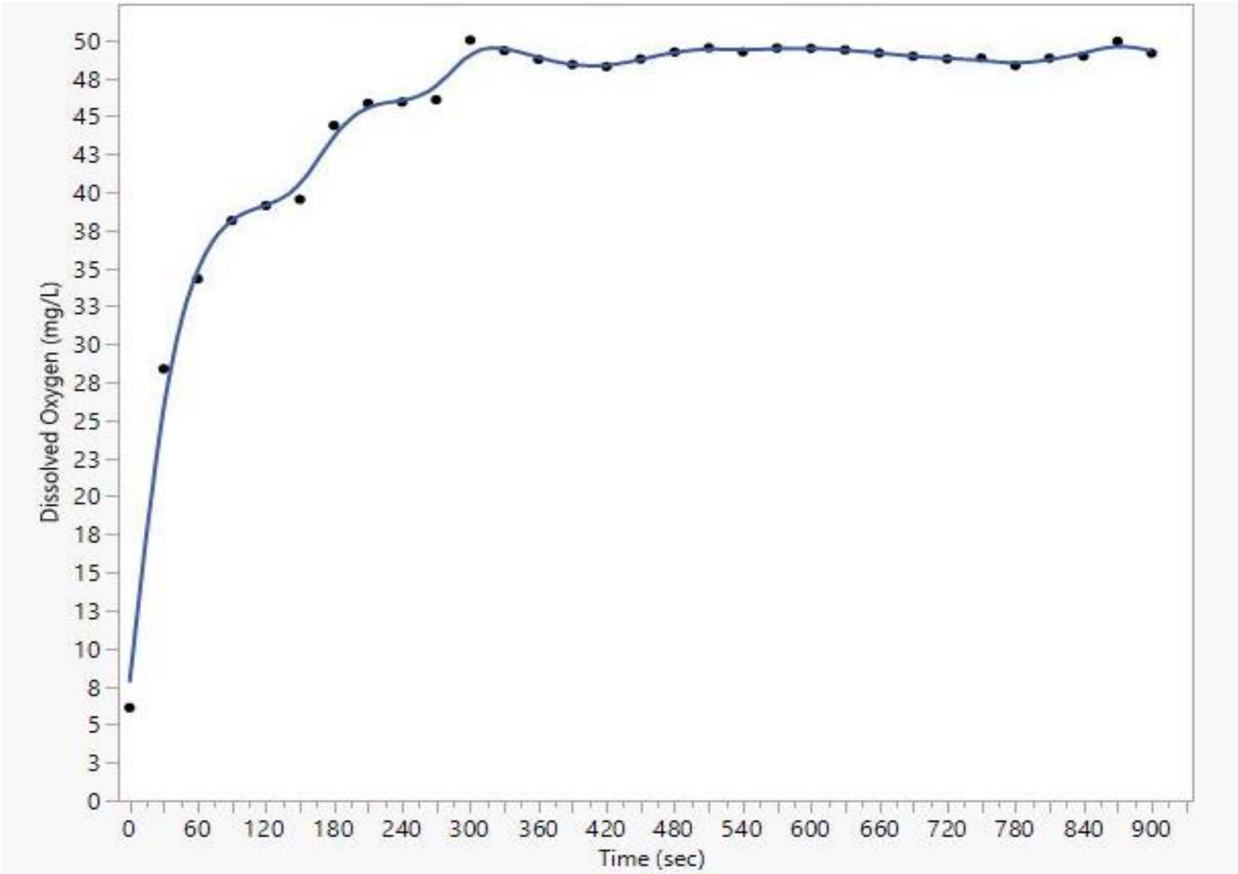

Figure 3.6: 2022 dissolved oxygen (mg/L) measurements to produce oxygenated nanobubble water over nine hundred seconds using the Rapid Waters Technologies (Grand Rapids, MI) unit. Dissolved oxygen concentration was taken at 15-second intervals.

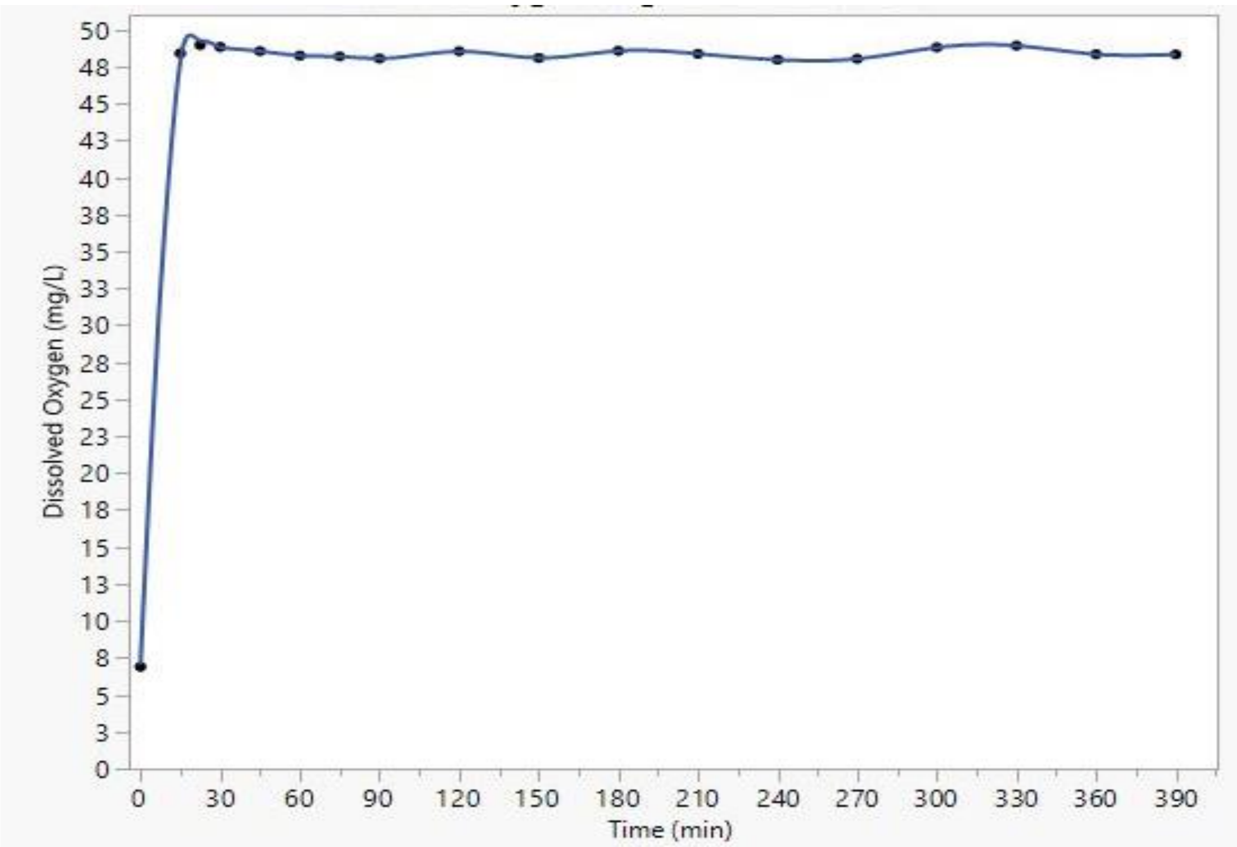

Figure 3.7: 2022 dissolved oxygen (mg/L) measurements to produce oxygenated nanobubble water over 390 minutes using the Rapid Waters Technologies (Grand Rapids, MI) unit. Dissolved oxygen concentration was taken at 15-minute intervals. The unit was turned off for 30 minutes, and the measurements after this show the stability of oxygenated nanobubbles in a deionized solution.

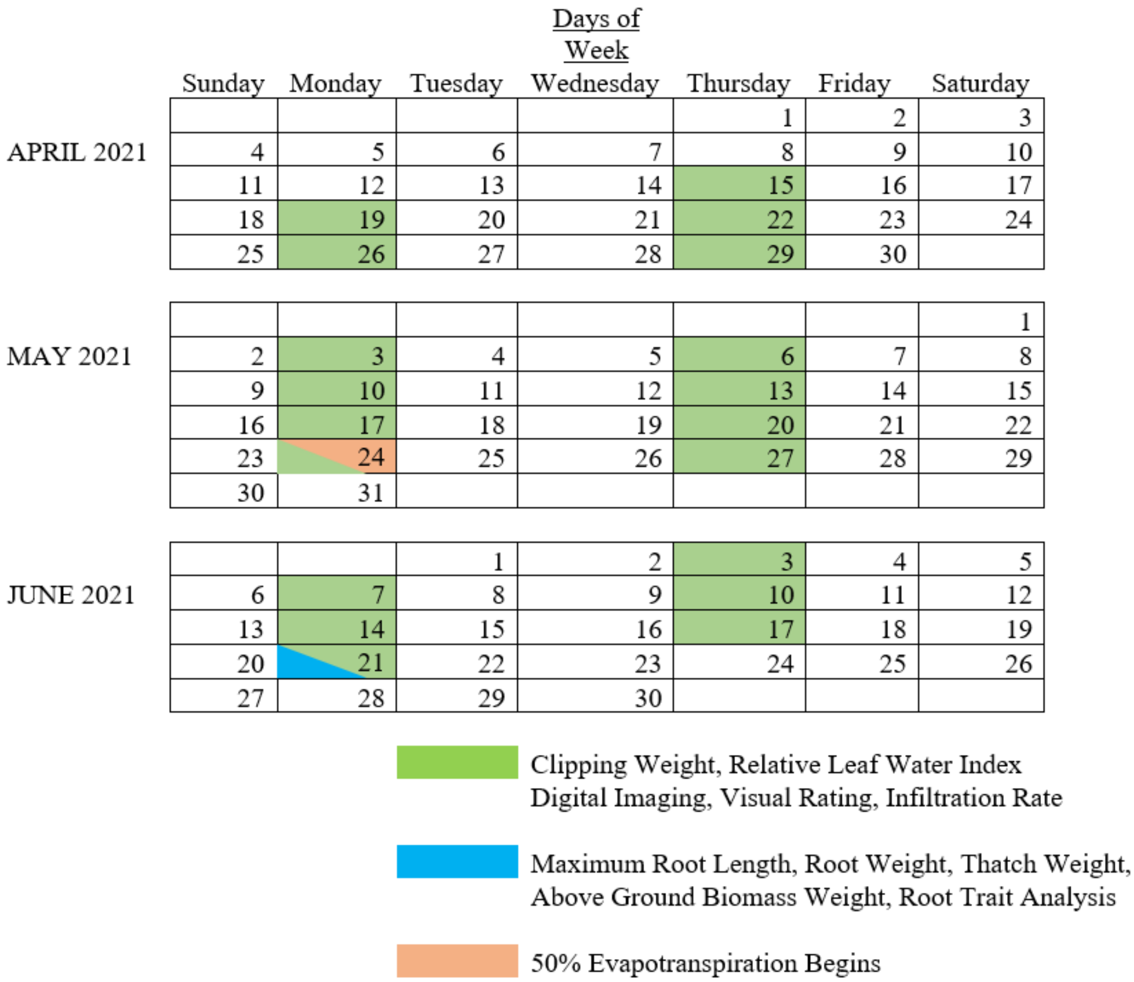

Figure 3.8: 2021 measurement schedule

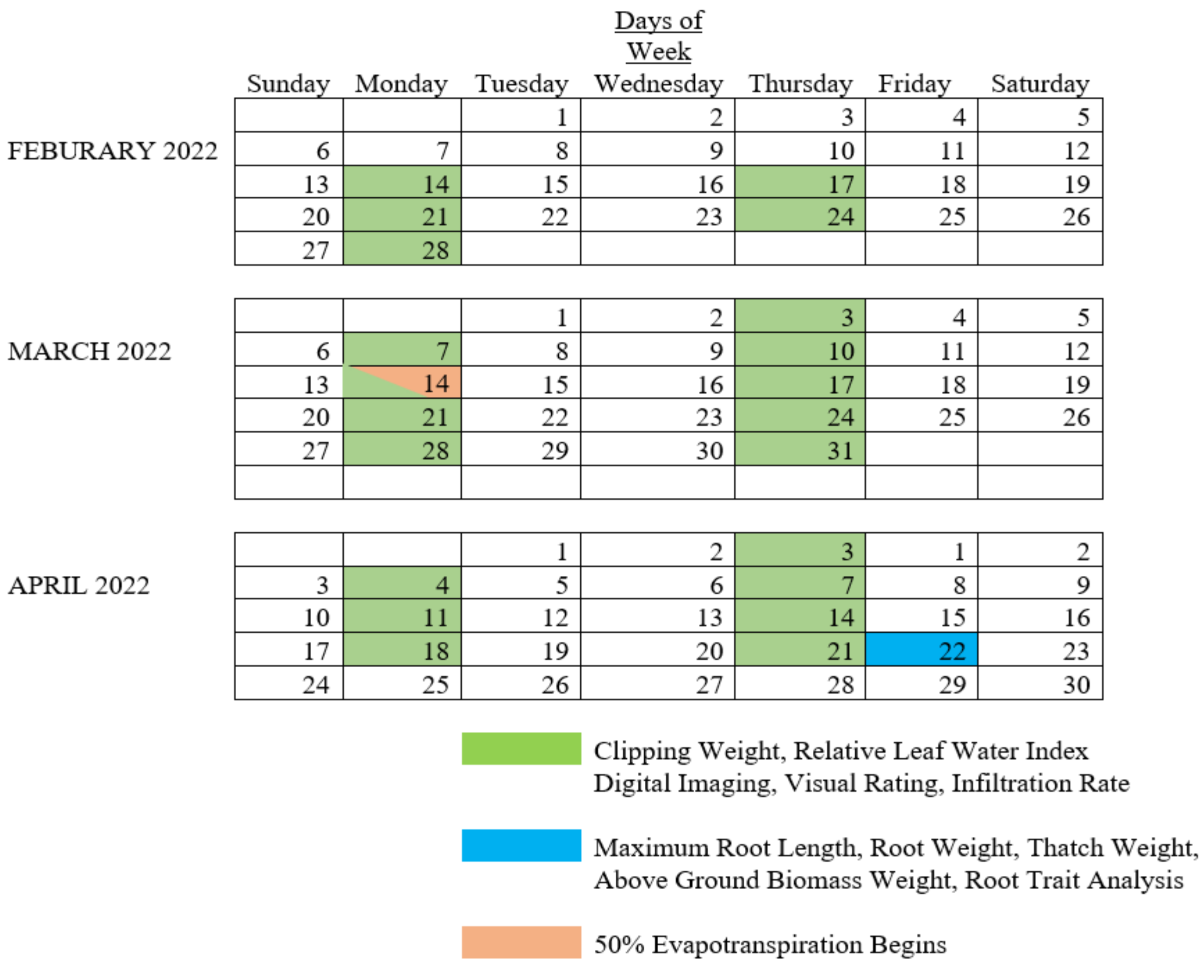

Figure 3.9: 2022 measurement schedule

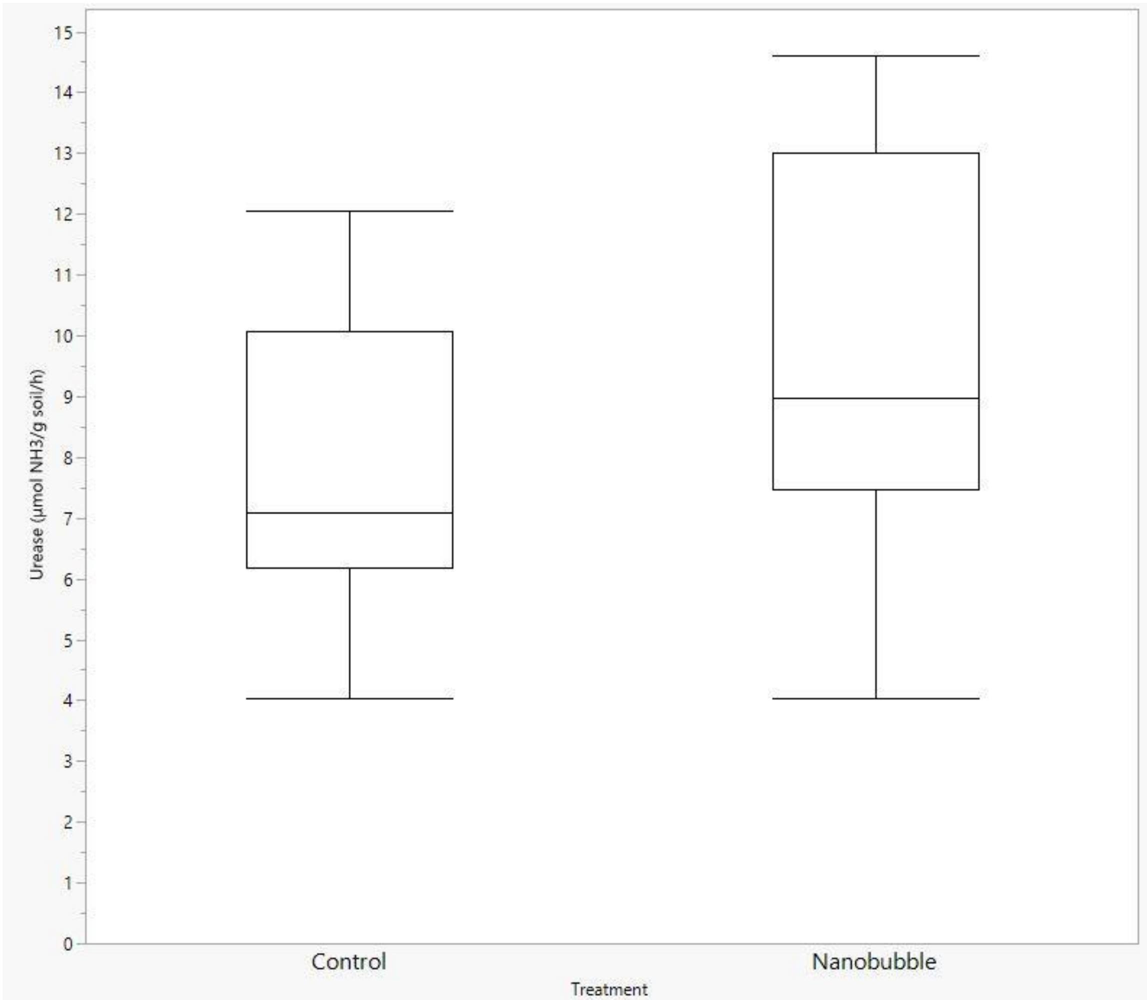

Figure 3.10: Urease activity (μmol NH_3 g^{-1} soil h^{-1}) in response to treatment with oxygenated nanobubble or tap water. The treatments did not significantly affect urease activity.

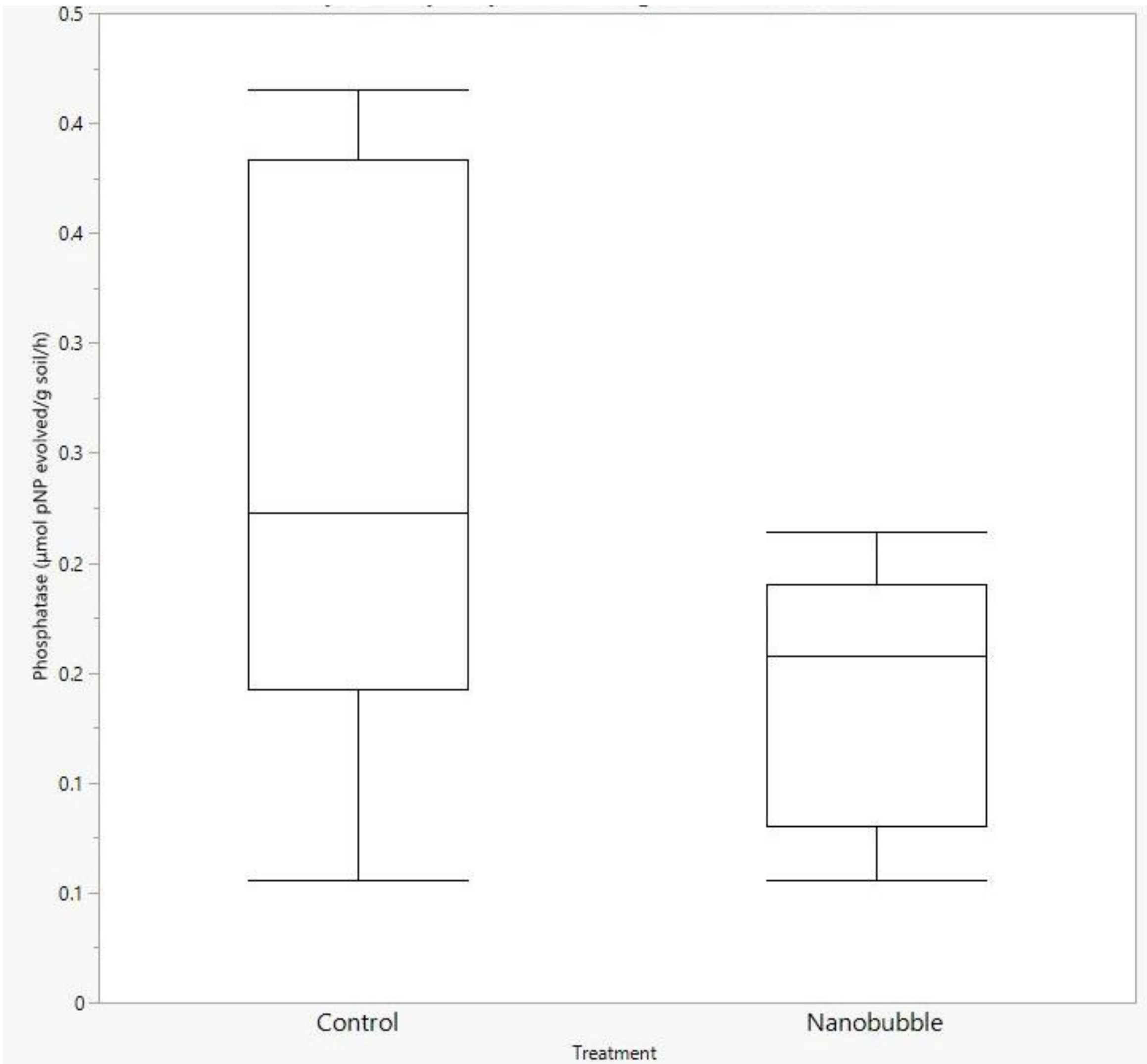

Figure 3.11: Phosphatase activity (μmol pNP g^{-1} soil h^{-1}) in response to treatment with oxygenated nanobubble water or tap water. The treatments did not significantly affect phosphatase activity.

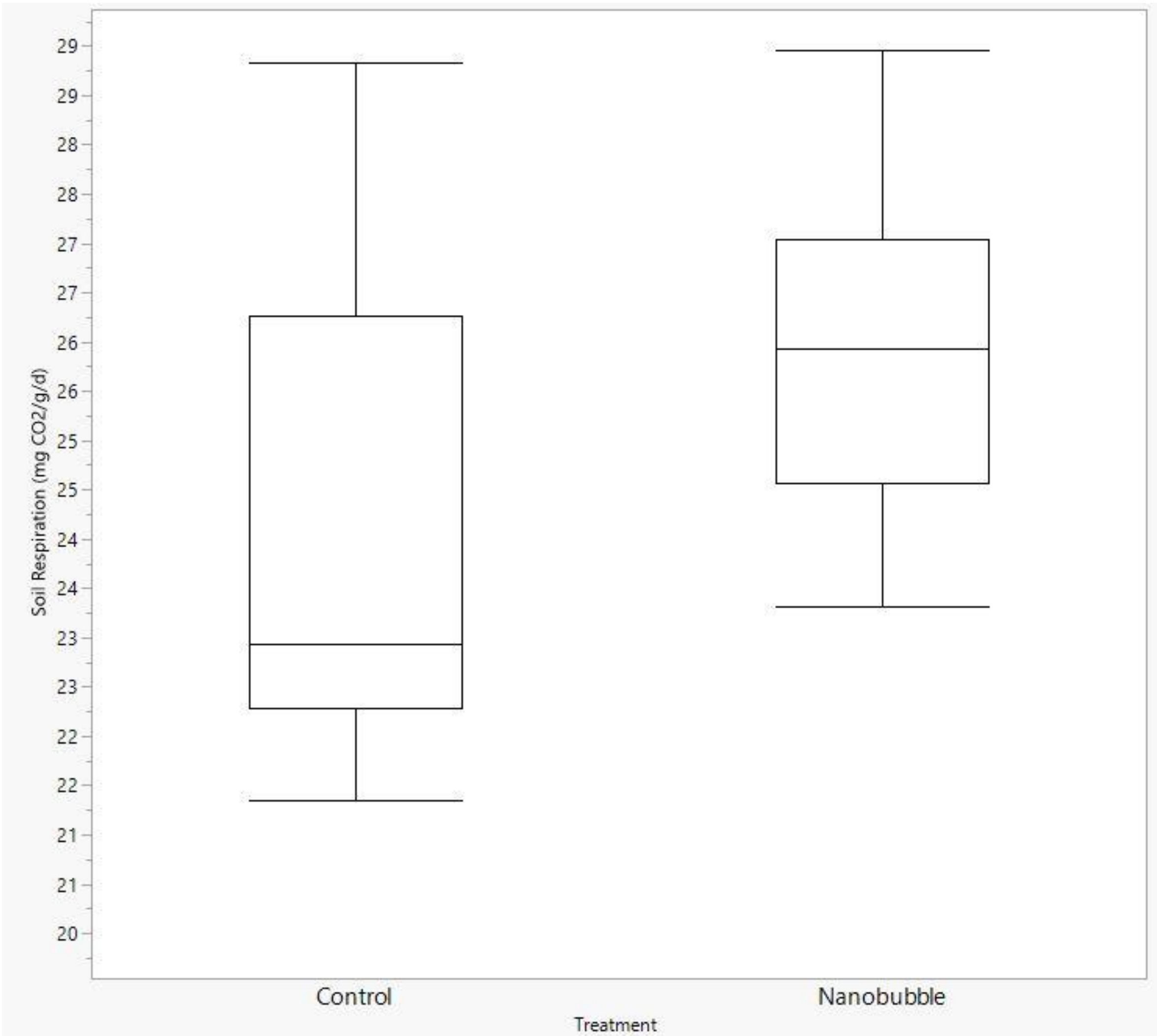

Figure 3.12: Soil respiration (mg CO_2 g^{-1} d^{-1}) in response to treatment with oxygenated nanobubble water or tap water. The treatments did not significantly affect soil respiration.

Table 3.1: Dissolved oxygen (mg/L) and temperature (℃) taken during the 2022 and 2023 greenhouse trials using the Rapid Waters Technologies (Grand Rapids, MI) nanobubbler showing the loss in dissolved oxygen when transferring oxygenated nanobubble water from container to beaker and then to be applied directly to the treatment pots for application.

Dissolve oxygen of deionized water in a tank	Dissolved oxygen of oxygenated nanobubble water in the mix tank	Dissolved oxygen of oxygenated nanobubble water in a beaker	Dissolved oxygen of oxygenated nanobubble water at application to pots	Temperature
7.10 mg/L	48.15 mg/L	34.43 mg/L	24.53 mg/L	28.5 ℃

Table 3.2: ANOVA table for clipping weight, relative water content, turf quality (% area greenness), and visual rating (1-9) turf quality

Year		Main effect *p*-value (α=0.05)			
		Response Parameters			
		Clipping Weight	Relative Leaf Water Content	Turf Quality (% area Greeness)	Turf Quality (Visual rating)
2021	Subtreatment (ST)	0.0007	0.0233	<0.0001	<0.0001
	Treatment (T)	0.0097	<0.0005	0.1715	0.0044
	ST*T	0.0666	0.0282	0.5351	0.0284
	Sampling Date*T	0.5755	0.0358	0.7275	0.0192
2022	Subtreatment (ST)	0.4738	0.5326	0.6786	0.6180
	Treatment (T)	0.0012	0.0650	0.0449	0.4783
	ST*T	0.0490	0.5540	0.9305	0.4855
	Sampling Date*T	1.0000	1.0000	0.1770	1.0000

Table 3.3: Mean clipping weight (g), relative water content (%), turf quality (% area greenness), and visual rating (1-9 scale) in response to two treatments with two sub-treatments in bermudagrass. Numbers with the same letter suffixes are not significantly different.

Response Parameter	Treatment	2021	2022
Clipping Weight (g)	Nanobubble	0.036 a	0.064 b
	Control	0.030 b	0.073 a
	(50% ET)	0.029 b	0.068 a
	(100% ET)	0.037 a	0.067 a
	(50% ET)*Nanobubble	0.030 b	0.062 b
	(100% ET)*Nanobubble	0.043 a	0.066 b
	(50% ET)* Control	0.028 b	0.077 a
	(100% ET)*Control	0.032 b	0.070 ab
Relative Water Content (%)	Nanobubble	71.8 b	94.5 a
	Control	75.5 a	93.2 a
	(50% ET)	72.4 b	94.1 a
	(100% ET)	74.8 a	93.6 a
	(50% ET)*Nanobubble	71.7 b	94.5 a
	(100% ET)*Nanobubble	71.8 b	94.5 a
	(50% ET)* Control	73.1 b	93.6 a
	(100% ET)*Control	77.8 a	92.7 a
Turf Quality Digital Imaging Analysis (% area greenness)	Nanobubble	72.9 a	85.4 b
	Control	70.8 a	86.3 a
	(50% ET)	65.8 b	85.8 a
	(100% ET)	77.9 a	86.0 a
	(50% ET)*Nanobubble	66.3 b	85.3 a
	(100% ET)*Nanobubble	79.4 a	85.5 a
	(50% ET)* Control	65.2 b	86.3 a
	(100% ET)*Control	76.3 a	86.4 a
Visual Rating Turf Quality (1-9 scale)	Nanobubble	6.42 a	8.70 a
	Control	6.12 b	8.70 a
	(50% ET)	5.69 b	8.60 b
	(100% ET)	6.85 a	8.79 a
	(50% ET)*Nanobubble	5.95 b	8.61 a
	(100% ET)*Nanobubble	6.88 a	8.78 a
	(50% ET)*Control	5.42 c	8.60 a
	(100% ET)*Control	6.81 a	8.79 a

Table 3.4: ANOVA table of digital imagery turf quality

Response parameters	Main effect *p*-value (α=0.05)		
	2021		
	Sub-Treatment	Treatment	ST*T
Turf Quality (% area greenness)	0.0915	0.9361	0.6666

Table 3.5: Mean turf quality through digital imaging analysis (% area greenness) in response to two treatments with two sub-treatments in Tifeagle bermudagrass for the 2023 soil health trial. Numbers with the same letter suffixes are not significantly different.

Response Parameter	Treatment	2023
Turf Quality in Digital Imaging Analysis (% area greenness)	Nanobubble	52.62 a
	Control	52.94 a
	(50% ET)	49.41 a
	(100% ET)	56.16 a
	(50% ET)*Nanobubble	48.40 a
	(100% ET)*Nanobubble	56.85 a
	(50% ET)* Control	50.42 a
	(100% ET)*Control	55.47 a

Table 3.6: ANOVA table for maximum root length, root weight, thatch weight, and above-ground shoot weight

Response parameters	Main effect *p*-value (α=0.05)					
	2021			2022		
	Sub-Treatment	Treatment	ST*T	Sub Treatment	Treatment	ST*T
Maximum Root Length	0.3590	0.1609	0.3590	0.1881	0.0257	0.1448
Root Weight	0.6390	0.6764	0.0186	0.1643	0.5794	0.0854
Thatch Layer Weight	0.8797	0.0217	0.8270	0.6883	0.9120	0.7147
Shoot Weight	0.9459	0.4452	0.9293	0.0147	0.6634	0.1163

Table 3.7: Mean maximum root length (cm), root weight (g), thatch weight (g), and above-ground shoot weight (g) in response to two treatments with two sub-treatments in Tifeagle bermudagrass. Numbers with the same letter suffixes are not significantly different.

Response Parameter	Treatment	2021	2022
Maximum Root Length (cm)	Nanobubble	15.7 a	11.9 b
	Control	14.5 a	14.2 a
	(50% ET)	15.4 a	13.7 a
	(100% ET)	14.7 a	12.4 a
	(50% ET)*Nanobubble	15.7 a	11.8 a
	(100% ET)*Nanobubble	15.7 a	11.9 a
	(50% ET)* Control	15.2 a	15.5 a
	(100% ET)*Control	13.7 a	12.6 a
Root Weight (g)	Nanobubble	0.729 a	0.339 a
	Control	0.691 a	0.388 a
	(50% ET)	0.731 a	0.428 a
	(100% ET)	0.689 a	0.299 a
	(50% ET)*Nanobubble	0.870 a	0.484 a
	(100% ET)*Nanobubble	0.588 a	0.193 a
	(50% ET)* Control	0.593 a	0.371 a
	(100% ET)*Control	0.790 a	0.405 a
Thatch Weight (g)	Nanobubble	30.1 b	16.9 a
	Control	39.0 a	17.2 a
	(50% ET)	34.3 a	16.7 a
	(100% ET)	34.8 a	17.5 a
	(50% ET)*Nanobubble	30.2 a	16.9 a
	(100% ET)*Nanobubble	29.9 a	17.0 a
	(50% ET)* Control	38.3 a	16.4 a
	(100% ET)*Control	39.6 a	18.0 a
Shoot Weight (g)	Nanobubble	2.64 a	3.02 a
	Control	2.81 a	2.97 a
	(50% ET)	2.72 a	3.18 a
	(100% ET)	2.73 a	2.81 b
	(50% ET)*Nanobubble	2.62 a	3.32 a
	(100% ET)*Nanobubble	2.66 a	2.73 b
	(50% ET)*Control	2.81 a	3.04 ab
	(100% ET)*Control	2.81 a	2.89 ab

Table 3.8: ANOVA table of root growth parameters

Response parameters	Main effect *p*-value (α=0.05)					
	2021			2022		
	Sub-Treatment	Treatment	ST*T	Sub Treatment	Treatment	ST*T
Average Root Width	0.2623	<0.0001	0.1629	0.1159	0.1866	0.4584
Max. Number of Roots	0.8974	0.0900	0.3127	0.5339	0.4818	0.2714
Med. Number of Roots	0.8119	0.0366	0.9367	0.4739	0.6652	0.4185
Number of Connected Components	0.7633	0.0963	0.0748	0.8585	0.6569	0.5168

Table 3.9: Mean root growth parameters in response to two treatments with two sub-treatments in TifEagle bermudagrass. Numbers with the same letter suffixes are not significantly different.

Response Parameter	Treatment	2021	2022
Average Root Width (cm)	Nanobubble	0.031 a	0.025 a
	Control	0.028 a	0.025 a
	(50% ET)	0.029 a	0.025 a
	(100% ET)	0.030 a	0.025 a
	(50% ET)*Nanobubble	0.031 a	0.026 a
	(100% ET)*Nanobubble	0.031 a	0.025 a
	(50% ET)* Control	0.027 b	0.025 a
	(100% ET)*Control	0.028 b	0.024 a
Maximum Number of Roots (n)	Nanobubble	25.1 a	19.4 a
	Control	22.3 a	17.7 a
	(50% ET)	23.8 a	19.3 a
	(100% ET)	23.6 a	17.8 a
	(50% ET)*Nanobubble	26.0 a	18.8 a
	(100% ET)*Nanobubble	24.2 a	20.0 a
	(50% ET)* Control	21.6 a	19.8 a
	(100% ET)*Control	23.0 a	15.6 a
Median Number Of Roots (n)	Nanobubble	18.6 a	13.7 a
	Control	15.7 b	12.8 a
	(50% ET)	17.3 a	14.0 a
	(100% ET)	17.0 a	12.5 a
	(50% ET)*Nanobubble	18.8 a	13.6 a
	(100% ET)*Nanobubble	18.4 a	13.8 a
	(50% ET)* Control	15.8 a	14.4 a
	(100% ET)*Control	15.6 a	11.2 a
Number of Connected Components (n)	Nanobubble	63.2 a	53.8 a
	Control	50.9 a	50.8 a
	(50% ET)	58.1 a	52.9 a
	(100% ET)	56.0 a	51.7 a
	(50% ET)*Nanobubble	57.6 a	52.2 a
	(100% ET)*Nanobubble	68.8 a	55.4 a
	(50% ET)* Control	58.6 a	53.6 a
	(100% ET)*Control	43.2 a	48.0 a

Table 3.10: ANOVA table of infiltration rate

Response parameters	Main effect *p*-value (α=0.05)			
	2021		2022	
	Sampling Date	Treatment	Sampling Date	Treatment
Infiltration Rate	0.0926	<0.0001	<0.0001	<0.0001

Table 3.11: Mean infiltration rate (mm^3/ sec) in response to two treatments in Tifeagle bermudagrass. Numbers with the same letter suffixes are not significantly different.

Response Parameter	Treatment	2021	2022
Infiltration Rate (mm^3/sec)	Nanobubble	18.5 b	7.79 b
	Control	24.5 a	9.29 a

Table 3.12: Mean soil health parameters in response to two treatments with two sub-treatments in Tifeagle bermudagrass. Numbers with the same letter suffixes are not significantly different.

Response Parameter	Treatment	2023
Soil respiration (mg CO_2 g^{-1} soil d^{-1})	Nanobubble	26.51 a
	Control	25.22 a
	(50% ET)	26.50 a
	(100% ET)	25.23 a
	(50% ET)*Nanobubble	26.32 a
	(100% ET)*Nanobubble	26.70 a
	(50% ET)* Control	26.68 b
	(100% ET)*Control	23.76 b
Urease activity (µmol NH_3 g^{-1} soil h^{-1})	Nanobubble	9.55 a
	Control	7.75 a
	(50% ET)	8.74 a
	(100% ET)	8.56 a
	(50% ET)*Nanobubble	9.02 a
	(100% ET)*Nanobubble	10.07 a
	(50% ET)* Control	8.46 a
	(100% ET)*Control	7.04 a
Phosphatase activity (µmol pNP g^{-1} soil h^{-1})	Nanobubble	0.172 a
	Control	0.243 a
	(50% ET)	0.228 a
	(100% ET)	0.187 a
	(50% ET)*Nanobubble	0.236 a
	(100% ET)*Nanobubble	0.109 a
	(50% ET)* Control	0.221 a
	(100% ET)*Control	0.265 a

Table 3.13: Mixed model analysis of enzyme activities and soil respiration in TifEagle bermudagrass treated with nanobubble or control water

Response variables	Main effect *p*-value (α=0.05)		
	2023		
	Sub-Treatment	Treatment	ST*T
Soil respiration ($mg\ CO_2\ g^{-1}\ soil\ d^{-1}$)	0.5183	0.5121	0.4041
Urease activity ($\mu mol\ NH_3\ g^{-1}\ soil\ h^{-1}$)	0.9052	0.2587	0.4278
Phosphatase activity ($\mu mol\ pNP\ g^{-1}\ soil\ h^{-1}$)	0.4852	0.2469	0.1662

CHAPTER 4

A FIELD STUDY ON THE EFFECT OF OXYGENATED NANOBUBBLE WATER ON SOIL HEALTH AND TURFGRASS GROWTH[2]

[2] Molini, M., Habteselassie, M., Jespersen, D., and Bahri, B. To be submitted to *Applied Soil Ecology*

ABSTRACT

The need to maintain high turf quality in golf courses often leads to extensive use of inputs that can have negative financial and environmental consequences. The potential benefits of oxygenated nanobubble water can help reduce inputs by improving turfgrass growth and soil health, but there has not been any research to evaluate its applications. A 5-month field study was conducted over two years in Johns Creek, GA to evaluate the impacts of oxygenated nanobubble water on turf growth and quality as well as soil biological health. Field plots under randomized complete block design were assigned two treatments: irrigation with nanobubble water vs pond water. Shoot and root growth parameters were measured regularly to evaluate turf growth whereas turf quality was evaluated via digital image analysis and visual rating. Soil health was assessed via microbial abundance, inorganic nitrogen content, enzyme assays, and soil respiration measurements. Average root width from digital root scanning showed significance in 2021, with nanobubble treatment yielding a higher mean than control, but these results were not consistent among trials. There were significant treatment effects on root weight in the 2022 trial, as the nanobubble treatment yielded a higher mean than the control, but these results were not consistent among trials too. There were no significant treatment effects on soil respiration, urease, and phosphatase enzyme assays, inorganic nitrogen content, or microbial abundance. Overall, the use of oxygenated nanobubble water did not impact turf or soil biological health parameters probably due to the short residence time of the bubbles.

INTRODUCTION

As a result of the pressure in maintaining high turf quality under climatic and management-induced stresses, superintendents rely on extensive use of inputs to maintain their golf courses (Strandberg et al., 2012) Such practices entail both financial and environmental costs that can be unsustainable in the long term. As such, there is a need to explore new technologies that can replace or reduce the need for extensive use of inputs. One such technology is oxygenated nanobubble technology.

Oxygenated nanobubble technology produces pockets of nano-sized oxygen-filled cavities in a liquid. One method in the development of oxygenated nanobubbles includes pushing compressed oxygen gas through a nano-sized membrane, producing as high as hundred million to ten trillion bubbles per milliliter of liquid, with bubbles below 1000 nm in size (Atkinson et al., 2019; Phan et al., 2020). Oxygenated nanobubbles have long-term stability in water, a negatively charged surface at the liquid-air interface, and high solubility in liquids. The use of oxygenated nanobubble water increased yields, better water-use efficiency, and better fruiting quality in cucumber, tomato, and corn agricultural systems (Baram, 2022). However, there are limited studies done on the use of oxygenated nanobubble water in a turfgrass system, as this technology is still in its infancy (Patel, 2021). The impact of oxygenated nanobubble water on turfgrass soil microbial communities is unknown as well.

There have been several studies on the use of oxygenated nanobubble water to mitigate oxygen deficiency in the rhizosphere (Lei et al., 2016). Low oxygen supply or poor soil aeration restricts root growth in soils that have been compacted or flooded (Huang et al., 1998). In addition, it had previously been reported that roots had lower density and reduced distribution under low oxygen conditions (O'Neil & Carrow, 1983). These problems in the rhizosphere can be improved through better aeration, where oxygen can be directly infused into irrigation water for use in the root zone of the plant. Some of the reported benefits associated with improved aeration are enhanced root respiration, better photosynthetic rate, accelerated fertilizer absorption, accelerated crop water absorption, improved performance of crop yield, higher irrigation water use efficiency, and improved crop quality (Zhou, 2019).

Soil health has been defined as "the continued capacity of soil to function as a vital living system, within an ecosystem and land use boundaries, to sustain biological productivity, maintain the quality of air and water environment, and promote plant, animal, and human health" (Lehman et al., 2015). Organic matter decomposition, nutrient cycling, disease suppression, and other ecosystem services in a turfgrass system are driven by soil microorganisms, which constitute the soil's biological health (Doran & Zeiss, 2000; Barrios, 2007; van der Heijden et al., 2008). Microbial decomposers are essential in the breakdown of organic matter into available nutrients that can be used by plants and are critical in use by turfgrasses (van der Heijden et al., 2008; Barrios, 2007, Steinke & Ervin et al., 2015). Microorganisms play a key role in providing ecosystems functions provided in healthy soils, including the nutrient provision and cycling, pest and pathogen protection, production of growth factors, water availability, decreased erosion, and increased water infiltration (Lehman et al., 2015; Kumar & Verma, 2019). Using oxygenated nanobubble water can have the potential to enhance the functions of microorganisms in soils due to improved aeration.

Microbial abundance and activity are indicators of soil health and have previously been used to monitor microbial communities due to changes in land use and vegetation (Doran and

Zeiss et al., 2000). Soil enzymes are mainly produced by microorganisms and measuring their activity is, therefore, a proxy for measuring microbial activity (Burns et al., 2013). As such, they can be good indicators of soil biological health (Asadishad et al., 2017; Harris, 2009). Enzymes associated with the cycling of nitrogen and phosphorous, such as urease and phosphatase, are reflective of changes in soil biological health (Pascual, 2002). Soil organic matter is produced from the decomposition of dead plants and animal materials, which can recycle nutrients and produce carbon dioxide (Parkin et al., 2015). Soil respiration acts as a good indicator of the overall activity of the microbial community (Allison et al., 2008).

Oxygenated nanobubble technology is still in its infancy, and research into its application in turfgrass systems is lacking. The objective of this study was to determine the impacts of oxygenated nanobubble water on soil biological health, turf growth, and quality in a field study.

MATERIALS AND METHODS

Study site

The field trial was established in August 2020 on ultradwarf TifEagle bermudagrass on a putting green at the Rivermont Golf Club in Johns Creek (34.001242, -84.2647569) and monitored for two growing seasons. Total precipitation during the 2021 trial was 80.65cm and for the 2022 trial was 52.60 cm. The field trial was a two-year study with the treatment of oxygenated nanobubble water being applied in the growing season of each year, which was May 25th, 2021 to September 27th, 2021, May 31st, 2022, and August 30th, 2022. The average maximum temperature for 2021 was 32.67℃ and the average minimum temperature for 2021 was 20.07℃. The average maximum temperature for 2022 was 32.67℃ and the average minimum temperature for 2022 was 20.07℃. Further climate data can be found for 2021 in the appendix under Figure A.3 and 2022 under Figure A.4. Soil oxygen and temperature sensors (Apogee SO-110 Soil Response Thermistor Reference Oxygen Sensors, Apogee Instruments, Logan, UT) were placed at a depth of 10 cm (4") in each plot of the putting green. The range of soil oxygen during the growing season was 16 to 21 partial pressure (mV). pressures. The average soil temperature during the growing season for 2021-2022 was 17℃ to 36℃.

Experimental set-up

Each plot was 1.2m by 1.2m, with a 0.381cm buffer between them. The plots were arranged in a randomized complete block design with four replicates. The experimental design schematic is shown in Figure 4.1. The field plots were a putting green sandy soil with 3% organic matter and an average pH of 6.5. There were two treatments with four replications, resulting in a total of 8 field plots. The treatments were divided by the irrigation water type. The first treatment receives pond water *without* oxygenated nanobubbles (101,102,103,104), acting as a control for the study. The second treatment receives pond water with oxygenated nanobubbles incorporated (201,202,203,204). Samples were taken monthly during the growing season. The field sampling schedule can be referenced in Figure 4.2.

The nanobubbles were generated with a 50-gallon per minute Moleaer unit (Moleaer, Carson, CA) unit. A NorthStar 98-L, 12-volt sprayer was used for irrigation, delivering 20 L min^{-1} from a Cool Shot Plus drenching nozzle. Irrigation treatments were applied 3x wk^{-1} to replace 70% reference evapotranspiration. Total dissolved oxygen (DO, mg L^{-1}) in the irrigation water, before and after passing through spray nozzles, was recorded at each irrigation event with a DO meter (HI98193, Hannah Instruments).

Both treatment and control irrigation was applied based on general management practices, based on weather, and water needed. Fertilizers were applied weekly during the growing season, from April to November. The fertilizers were a combination of organic and synthetic, foliar feedings only. 45.4 grams to 68.0 grams of nitrogen was applied per week, using a fertilizer of (3.2 N-0.14 P- 1.7 K). In addition, Daconil was used in one instance for cream leaf spot of the plots. Lastly, additives like molasses, seaweed extract, and other microbial products were used to provide sustenance for microbes within the soil. The greens were maintained at 0.105 cm-0.120 cm heights during the growing season, raising the mowing height during dormant turfgrass periods for winter.

Field sampling for soil analysis

Field Samples were taken monthly during the growing season (Figure 4.2). Composite soil samples of 6 soil cores were collected at random from each plot using a soil auger. The soil auger collected samples that were 2.54 cm in diameter and 15.24 cm in depth. Soil sample collection was done while wearing gloves and with a soil auger that was sprayed with a general antibacterial disinfectant between each plot. Paper towels were used to remove the disinfectant from the probe after spraying. This step was repeated between each plot to prevent cross-contamination of soil microbes between field study plots. Soil samples were sealed in plastic bags and placed in a cooler, maintained at 4℃, with ice packs to preserve samples until they could be processed in Griffin, Georgia.

The samples were sieved through a 2-mm sieve to remove plant materials. Part of the sieved samples was then used to measure soil respiration, enzyme activities, and ammonium and nitrate concentrations. Samples were analyzed immediately or within two weeks of storage in a refrigerator at 3 ºC. Approximately 10 g of soil from each bag was also immediately frozen at -15 °C for later DNA extraction to determine microbial abundance via quantitative polymerase chain reaction. Soil moisture was determined gravimetrically. Moist soil was weighed on the same date as the extraction of the sample after having been sieved. Dry weights were obtained after drying in an oven at 100℃ for 24 h. The moisture content of all samples was calculated and further used to calculate the oven-dry soil weight (g) equivalent of the amount used for analysis.

Methods for determining soil moisture, enzyme activities, and soil respiration are described in Chapter 3, section Materials and Methods.

Inorganic N content (nitrate and ammonium) determination

For ammonium and nitrate analysis, KCl extraction from soil was conducted according to Habteselassie et al. (2006) in which a suspension of soil was prepared with a 1:5 ratio of soil and 2M KCl and shaken for 2 hrs, followed by filtration. The KCl extracts were sent to Waters Agricultural Laboratories (Camilla, Georgia) to analyze for ammonium and nitrate with a flow-injection autoanalyzer.

Quantitative polymerase chain reaction

Quantitative polymerase chain reaction (qPCR) was used to determine the abundance of total bacteria and total fungi. Using DNeasy PowerSoil DNA extraction kit (Qiagen, Germantown, MD), soil DNA was extracted from all the samples. Samples from July 2021, August 2021, July 2022, and August 2022 were used for qPCR. The reaction volume for qPCR was 20 µL containing 10 µL PowerUp SYBR Green Master Mix (ThermoFisher Scientific, Grand Island, New York), 2 µL DNA template, 1.0 µL of forward primers, 1.0 µL of reverse primers, and 6.0 µL nuclease-free PCR water; all reactions were run duplicate. The primers used for the total bacteria were EUB 338 (forward) and EUB 518 (reverse), and the primers used for total fungi were nu-SSU 0817 (forward) and nu-SSU 1196 (reverse). The serial dilutions of stock target organisms ranged from 30 to 3×10^5 copies of DNA per µL were prepared and were run in analytical triplicate for all assays. StepOne Software (Applied Biosystems) was used to analyze the generated qPCR data. Table 4.1 summarizes the target genes, amplicon lengths, primers, and thermal cycling conditions used to quantify total bacteria and total fungi. The qPCR reaction efficiencies and R2 values for standard curves generated by StepOne ranged from 80.68% to 118.502% and 0.952 to 0.998. The standard curves and equation were used to calculate the quantity of DNA in each soil sample.

$$\text{copies g}^{-1} \text{ soil} = (\text{x copies} / 2\ \mu\text{L}) \times (100\ \mu\text{L} / 0.25\ \text{g soil})$$

Field sampling for turf analysis

A soil core cylinder with dimensions of 36.83 cm in length and 5.08 cm in width was hammered into the ground using a rubber hammer to a depth of 15.24cm. This soil core was to be used for the physiological aspects of this field study. The soil core was done once per plot and placed and sealed in a plastic bag. Soil core samples were placed in a cooler, maintaining approximately 4℃. When back at the UGA Griffin campus, the soil core sample was refrigerated at 2.8℃. This soil core was dismantled to yield root weight, shoot weight, and root scan image analysis.

Shoot weight

From the soil core, a knife was used to remove the top 1cm of the soil core, thereby separating the grass from the soil cylinder. This grass was placed into a brown paper bag. These bags were then placed into an incubator at 70°C for 4 days or more. The samples were removed from the incubator and a 2-mm sieve was used to remove the sand from the grass, leaving us with only the grass sample. This grass sample was then weighed using a (Mettler AE 100) balance. This measurement retrieved the dry shoot weight.

Turf quality with digital image analysis & visual rating

For Digital imagery a Canon G9X digital camera with the custom settings of Iso: 400, Flower interface, F: 4.0, and custom white balance of 0 was used. A Lightbox is used to capture an image of the plot to ensure uniformity of lighting. One image was taken per pot. This was repeated through all plots in the Rivermont field trial. The images were run through Fiji ImageJ software as a percent green cover. This provides an objective assessment of the overall turf quality and quantitative data for future statistical analysis. From this, a percentage of the green cover area of greenness was determined (Jespersen et al., 2019).

A visual rating was assigned to each plot based on turfgrass quality. It was based on a scale of 1-9, using color, density, and uniformity to determine the rating, as described in (Krans & Morris, 2007).

Rooting traits & root weight analysis

A soil coring cylinder was hammered into the ground getting a soil core size of 15.24cm in length and 5.08cm in width. The top thatch layer and grass shoot biomass were removed from the core. The sample was washed thoroughly, and roots were removed from the sieve using tweezers. The samples were placed in an 80% ethanol solution until scanned.

The roots were selected based on being a representative sample of the soil core. These roots were placed on a plastic tray used for scanning through an Epson Scanner (Epson Perfection V550 Photo). The plastic tray was 30.48 cm in length, 21.59 cm in width, and 1.27cm in height to fit the scanner, as well as be able to sustain a thin layer of water. This thin layer of water was used to suspend the representative roots in an aqueous solution. The roots were placed in this DI water bath fully covering the plastic tray. Debris and organic matter were removed from the root samples, as to get a better indicator of the parameters being measured. The tray and root samples were scanned, producing a high-quality JPG image that was saved to the computer (Katuwal et al., 2020). These images were run through GIAroots digital imaging root analysis program to yield the average root width, the maximum number of roots, the median number of roots, network area, network length, network perimeter, network surface area, network volume, number of the connected component, and specific root length.

After the scanning process, the root samples were removed from the plastic scanning tray and placed into #1 coin envelopes (Quality Park, 2.25in X 3.5in) and an

incubator at 70°C for 4 days or more. After this period the root samples were removed from their envelopes and a (Mettler AE 100) balance was used to record this root dry weight.

Statistical analysis

Repeated measures analysis of variance (ANOVA) was carried out in testing the statistical significance of the effects of oxygenated nanobubble water on turf quality and indicators of soil health at a significance of 0.05 in JMP Pro 16 (SAS JMP, NC) over multiple sampling dates during the trials. Tukey's honest significant difference (HSD) test was used for mean separation when the treatment effect was significant.

RESULTS AND DISCUSSION

Microbial abundance

The mean total bacterial abundance for control was 1.5×10^7 copies g^{-1} soil and the mean total bacteria for treatment was 1.2×10^7 count g^{-1} soil. There were no significant treatment effects on total bacteria (Table 4.2 & Fig. 4.3). The mean total fungal abundance for control was 4.2×10^6 count g^{-1} soil and the mean total fungi for treatment was 2.3×10^6 count g^{-1} soil. There were no significant treatment effects on total fungi (Table 4.2 & Fig. 4.4). The dominance of bacteria over fungi in both treatment and control soil is not surprising, as bacteria are more abundant in highly managed systems. In addition, the dominance of bacteria over fungi can be attributed to the putting green being highly disturbed, mildly acidic, and having received frequent fertilizer applications. A potential reason for the lack of treatment effect could be that oxygen availability was not a limiting factor in this turfgrass system. There may have been another factor that could potentially have more influence over the soil microbial abundance than the oxygen deficiency in the soil. For instance, soil respiration and microbial abundance have previously been found to have a positive correlation with increased soil temperature and soil moisture. In a study using aerated irrigation on tomato plants, it was found that even with soil temperature being controlled, there was a positive correlation between soil oxygen concentration and microbial abundance (Zhu et al., 2019). This may be indicative that there are factors not considered in this study that may have more of an effect on total bacteria and total fungi, than the soil oxygen concentration.

Inorganic N content

The concentrations of ammonium and nitrate were determined as another way of determining the aeration status of the soil and as a reflection of the possible impact of oxygenated nanobubble water on the soil oxygen level. Nitrification, the process by which ammonium is oxidized to nitrate, is aerobic. The mean ammonium concentration for control was 24.95 mg NH_4 kg^{-1} soil and the mean ammonium concentration for treatment was 32.02 mg NH_4 kg^{-1} soil (Fig. 4.5). The mean nitrate concentration for control was 7.47 mg NO_3^{-1} kg^{-1} soil and the mean nitrate concentration for treatment was 8.23 mg NO_3^{-1} kg^{-1} soil (Fig. 4.6). There were no significant treatment effects on nitrate content and ammonium concentrations (Table 4.3).

Nitrification is inhibited if the soil is low in oxygen, as ammonia-based fertilizers break down in the soil to nitrate (Gelerter & Stowell, 2001). As a result, there may not have been a shortage of oxygen in the field trials due to no significant difference between treatment and control. With an adequate presence of oxygen already present in the soil, the addition of oxygenated nanobubble irrigation may have a limited effect on nitrification and the presence of nitrate and ammonium contents in the soil.

Soil respiration

The mean soil respiration for control in 2021 was 1.85 mg CO_2 g^{-1} soil d^{-1} and in 2022 was 1.85 mg CO_2 g^{-1} soil d^{-1} (Fig. 4.7). The mean soil respiration for treatment in 2021 was 1.88 mg CO_2 g^{-1} soil d^{-1} and in 2022 was 1.84 mg CO_2 g^{-1} soil d^{-1} (Fig. 4.7). There were no significant treatment effects on soil respiration (Table 4.4). Treatment applications did not significantly influence soil respiration rates in either trial. There were significant sampling date*treatment effects for 2021 and 2022 but did not give insight into why soil respiration did not yield significant treatment effects for both years (Fig. A.5 & Fig. A.6)

Soil oxygen showed significant treatment effects, with the control yielding a higher mean of 19.24 mV than the oxygenated nanobubble treatment of 19.01 mV (Tables 4.5 & 4.6). Even though there were significant treatment effects between these partial pressure readings, the difference in readings is small. The level of oxygen in nanobubble water decreased from 31.62 mg/L to 22.11 mg/L at the nozzle (Table 4.7). The loss of oxygen in the application of the oxygenated nanobubble water could be the reason that even with significant treatment effects on soil oxygen, the mean differences are close to each other (Fig. 4.8). It can even be hypothesized that when the oxygenated nanobubble treatment is supplemented into the soil, the oxygen is not staying in the soil and so may not have the chance to be used by the soil microbes. This may have resulted in a lack of treatment effect, due to oxygen not being added to the turfgrass system.

Enzyme activities

The mean urease activity for control in 2021 was 16.9 µmol NH_3 g^{-1} soil h^{-1} and in 2022 was 6.44 µmol NH_3 g^{-1} soil h^{-1} (Fig. 4.9). The mean treatment urease activity in 2021 was 15.7 µmol NH_3 g^{-1} soil h^{-1} and in 2022 was 6.16 µmol NH_3 g^{-1} soil h^{-1} (Fig. 4.9). There were no significant treatment effects on soil urease enzyme activity (Table 4.4). There were significant sampling date*treatment effects for the 2021 field trial but does not give insight into why the urease assay did not yield significant treatment effects for both trials (Table 4.4 & Fig. A.7). It can be noted that there is an overall decrease in urease activity from 2021 to 2022, but insight into what caused this decrease across trials is unknown. This decrease may stem from an external factor not recognized in these trials.

The mean phosphatase activity for control in 2021 was 0.606 µmol pNP g^{-1} soil h^{-1} and in 2022 was 0.188 µmol pNP g^{-1} soil h^{-1} (Fig. 4.10). The mean phosphatase activity for treatment in 2021 was 0.508 µmol pNP g^{-1} soil h^{-1} and in 2022 was 0.190 µmol pNP g^{-1} soil h^{-1} (Fig. 4.10). There were no significant treatment effects on soil phosphatase enzyme activity (Table 4.4). Furthermore, the sampling date did not significantly influence phosphatase activity for 2021 but did influence activity for 2022. There were no significant sampling date*treatment effects for the phosphatase activity for either trial. The phosphatase activity showed a large difference in results from 2021 to 2022. This resembles what was shown in the urease activity. This decrease between 2021 to the 2022 trials may stem from an external factor that may not be recognized within these trials.

Urease and phosphatase activity can be an indicator of change potentially brought about by the oxygenated nanobubble technology on the nitrogen cycle. The absence of any significant impact indicates that the treatment with oxygenated nanobubble water did not influence the cycling of nitrogen.

Urease and phosphatase enzymes behave differently than microorganisms as they are secreted in the rhizosphere and can exist in conditions that cannot be tolerated by microorganisms. In addition, because protein molecules create complexes with soil organic matter, they have been closely associated with the thatch layer in turf soils. In addition, it has been suggested that most of the variation in urease activity among soils can be accounted for by the quantity of soil organic matter, with SOM protecting soil urease from microbial degradation without disturbing enzyme activity (Torello & Wehner, 1983). Because thatch is an extensive layer of soil organic matter that resides on the soil surface, there is potentially a high level of urease occupying the thatch layer. As a result, sieving the soil sample to separate the plant material and contaminants from the soil might remove some elements that contribute to these enzymatic activities along with the thatch layer (Mueller & Kussow, 2015). This can potentially affect their activity during laboratory analysis. In addition, it is possible that the limiting factor for urease activity was not oxygen stress. Enzyme activities can potentially fluctuate in the upper layers of soil due to dramatic temperature changes, as opposed to deeper in the soil profile where temperatures remain cooler and steadier (Diera et al., 2020).

Turf growth and quality

There were no significant treatment effects on turf quality as % green cover (Table 4.8). The range of mean turf quality for 2021 was 84.7 to 84.9 percent green cover, while the range of mean turf quality for 2022 was 88.4 to 89.4 percent green cover (Table 4.9). There were no significant treatment effects on turf quality with visual rating too (Table 4.8). The visual rating mean score ranged from 8.75 to 8.77 for the 2021 trial and was 8.32 for both control and treatment in the 2022 trial (Table 4.9). There were no significant sapling date effects on either trial for either year. There was no significant treatment effect on shoot weight (Table 4.8). The mean shoot weights for control were 1.66 g in 2021 and 2.97 g in 2022 (Table 4.10). The mean shoot weights for oxygenated nanobubble treatment water were 1.76 g for 2021 and 2.81 g for 2022. There was an increase in shoot weights for 2022 than in 2021, but the reason why this was seen is not known. There were significant sampling date*treatment effects for the 2021 trial regarding turf shoot weight, but it does not give insight into why we did not see significant treatment effects for the 2021 trial (Fig. A.8).

It is possible that soil oxygen was not the limiting factor in this turfgrass system, and therefore there were no significant treatment effects on the turf growth or turf quality. For instance, mowing height, removal of grass clippings from a site, and the accessibility the turfgrass has to N, all play a role in turf quality and improved turf color in a golf course system (Kopp & Guilliard, 2002). There may be an external factor not recognized in this study that may be the limiting factor other than oxygen deficiency.

Root scan analysis and root growth parameters

There were significant treatment effects on average root width for the 2021 trial (Table 4.11). The control showed a lower average root width of 0.021 cm, while the nanobubble treatment showed an average root width of 0.022 centimeters (Table 4.12). There were no significant treatment effects on the average root width for the 2022 trial (Table 4.11). Although the average root width did show the significance for the 2021 trial, the difference in means is not very high. There were no significant treatment effects on the maximum number of roots (Table 4.11). The maximum number of root means ranged from 25.5 to 31.9 counts (n) (Table 4.12). There were significant sampling date*treatment effects for the maximum number of roots, but this does not give insight into why there were no significant treatment effects (Fig. A.9). There were no significant treatment effects on the median number of roots (Table 4.11). The median number of root means ranged from 18.6 to 23.3 counts (n) (Table 4.12). There were no significant treatment effects on the number of connected components (Table 4.11). The number of connected components means ranged from 94.5 to 102 counts (Table 4.12).

There was a significant treatment effect on root weight for the 2022 trial, as the nanobubble treatment yielded a higher mean root weight than the control. There was no significant treatment effect on root weight for the 2021 trial (Table 4.8). The mean root weight for the control was 0.097 g for 2021 and 0.107 g for 2022. The mean root weight for the oxygenated nanobubble treatment was 0.090 in 2021 and 0.135 in 2022. For neither the root weight nor the response parameters given by the root scan, there were no significant sampling date*treatment effects.

The inconsistent root weights and root widths across trials, in addition to the lack of significant treatment effects on any of the other response parameters given by the root scan, may mean that oxygen may not have been the limiting factor in these trials. The factor that affects the root weight and root width may be external to what has been considered in these trials and may be what is affecting these measurements.

Soil oxygen and soil temperature

The soil oxygen and soil temperature data can give insight into the influence of nanobubble water on soil oxygen levels over time. There was a significant treatment effect on soil oxygen, but no significant effect was seen for the time*treatment effects (Table 4.5). The control water treatment yielded higher soil oxygen (mV) with a mean average of 19.24 mV than the control, with nanobubble treatment yielding 19.01 mV (Table 4.6).

The soil temperature showed no significance in treatment, nor treatment*time interaction in the field site throughout the duration of the study (Table 4.6). The mean average for the nanobubble treatment was 19.96 °C and the mean average for the control was 19.90°C (Table 4.6 & Fig. 4.11).

SUMMARY and CONCLUSIONS

Overall, oxygenated nanobubble water did not significantly impact biological soil health parameters (soil respiration and enzyme activities). In the context of root parameters, there was no significant effect of treatment on the maximum number of roots, median number of roots, number of connected components, or specific root length. There was a significant treatment effect on average root width for 2021, but this was not repeated in 2022. There was no significant treatment effect on shoot weight. There was a significant treatment effect on root weight in 2022, but this was not replicated in 2021. There was no treatment effect on turf quality as determined through digital image analysis and visual rating. There was a significant treatment effect on the soil oxygen based on in-situ sensors. Although the soil oxygen did show significant treatment effects, the difference was small. The lack of consistent treatment effect of oxygenated nanobubble water on soil biological health and turf growth and quality parameters suggest that the system was not limited by oxygen availability. Continuous monitoring of soil oxygen data also suggested that the oxygen introduced into the soil in nanobubbles was not staying there as there was little difference in soil oxygen concentration between the control and treatment plots. This might account for the lack of consistent treatment effects as well. There may have been external factors that were not considered in this study that had more of an effect on the turfgrass growth and quality, as well as the soil microbial activity and abundance.

This study presents some insight into the dynamics between oxygenated nanobubble water and soil microbial communities and turfgrass for a golf course. The practical importance of the study is to provide information to golf course superintendents and turfgrass managers about new technologies that are marketed to them to enable them to make informed decisions.

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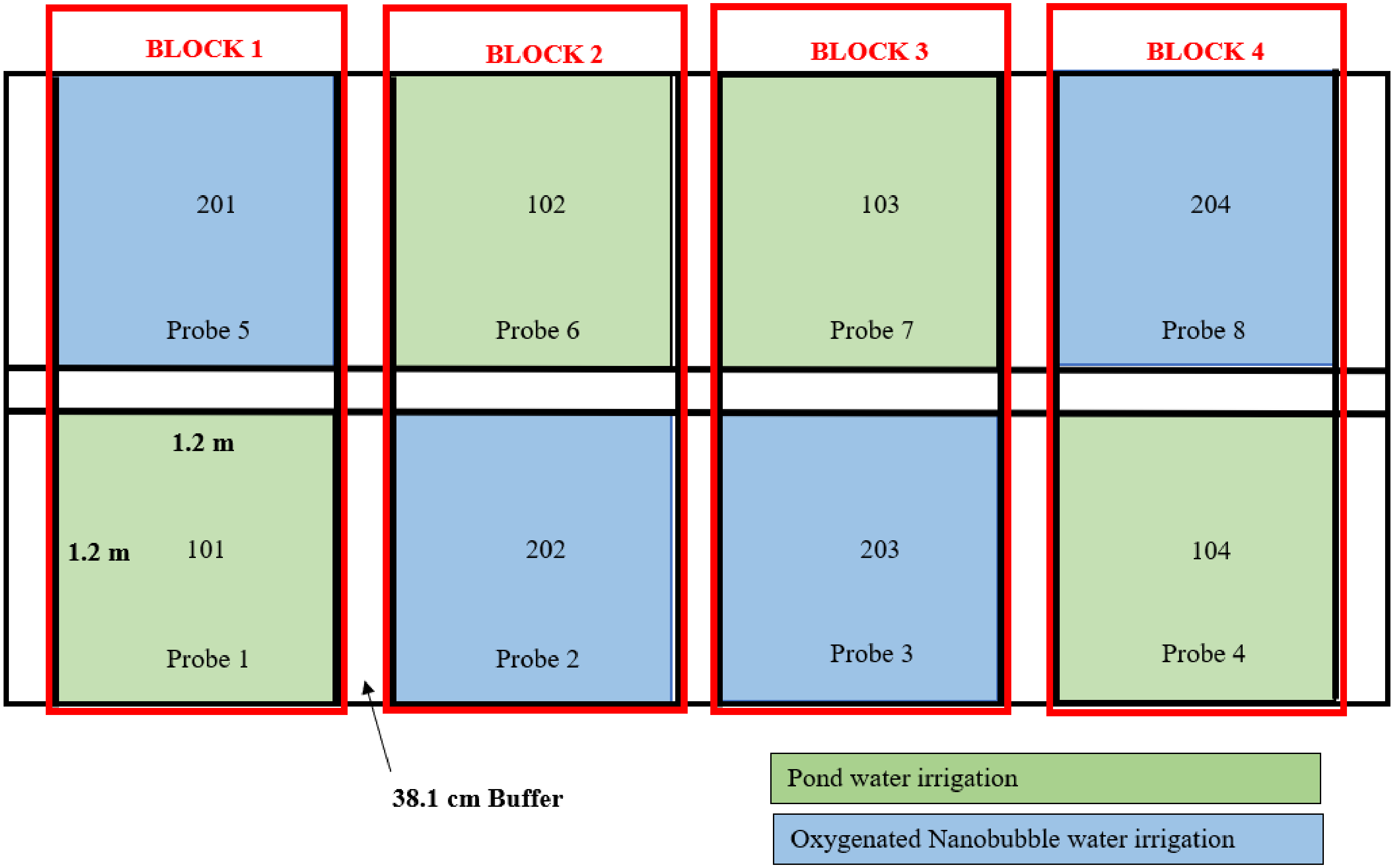

Figure 4.1: Design of experimental field plots in Rivermont Golf Club

2021

March	April	May	June	July	August	September	October
		5/25/2021	6/24/2021	7/27/2021	8/24/2021	9/27/2021	

2022

March	April	May	June	July	August	September	October
	4/12/2022	5/31/2022	6/28/2022	7/29/2022	8/30/2022		

Field Sampling at Rivermont

Pre-Treatment Sampling

Figure 4.2: Rivermont Golf Club field sampling schedule 2021-2022

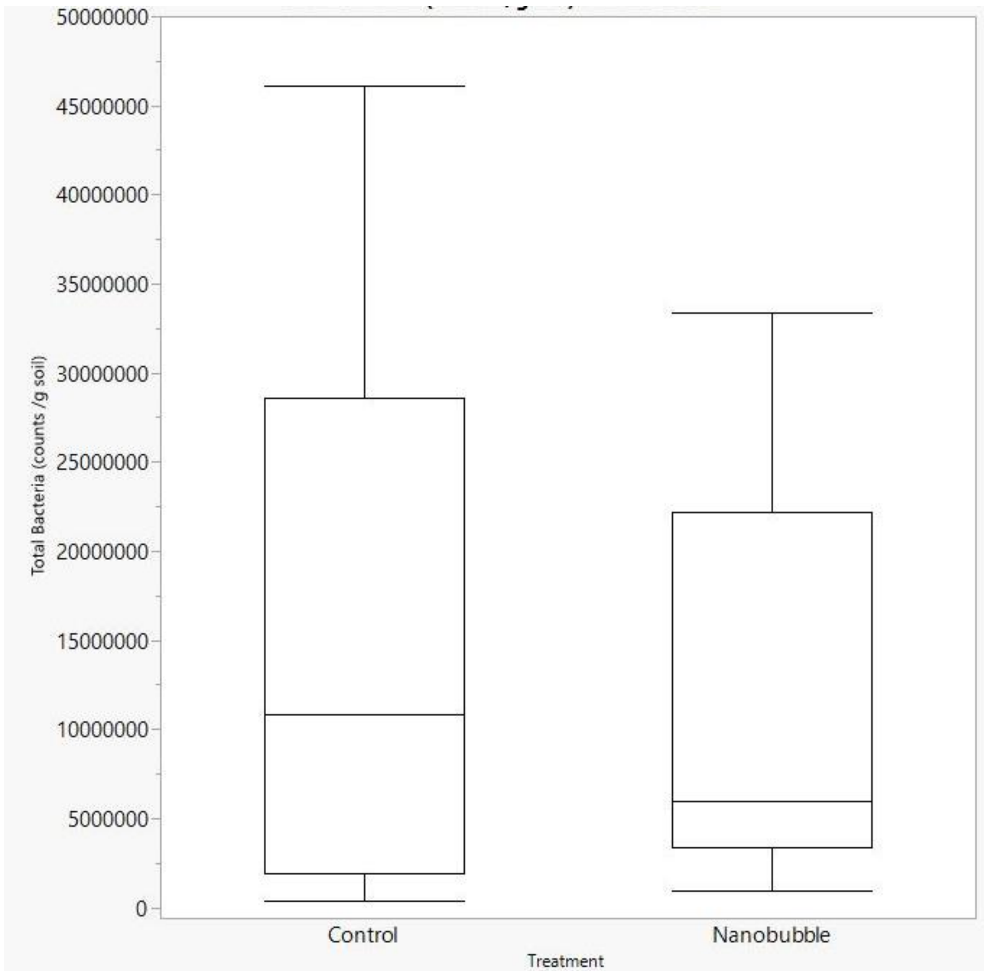

Figure 4.3: Total bacteria abundance (count g^{-1} soil) in response to treatment with oxygenated nanobubble water or control pond water.

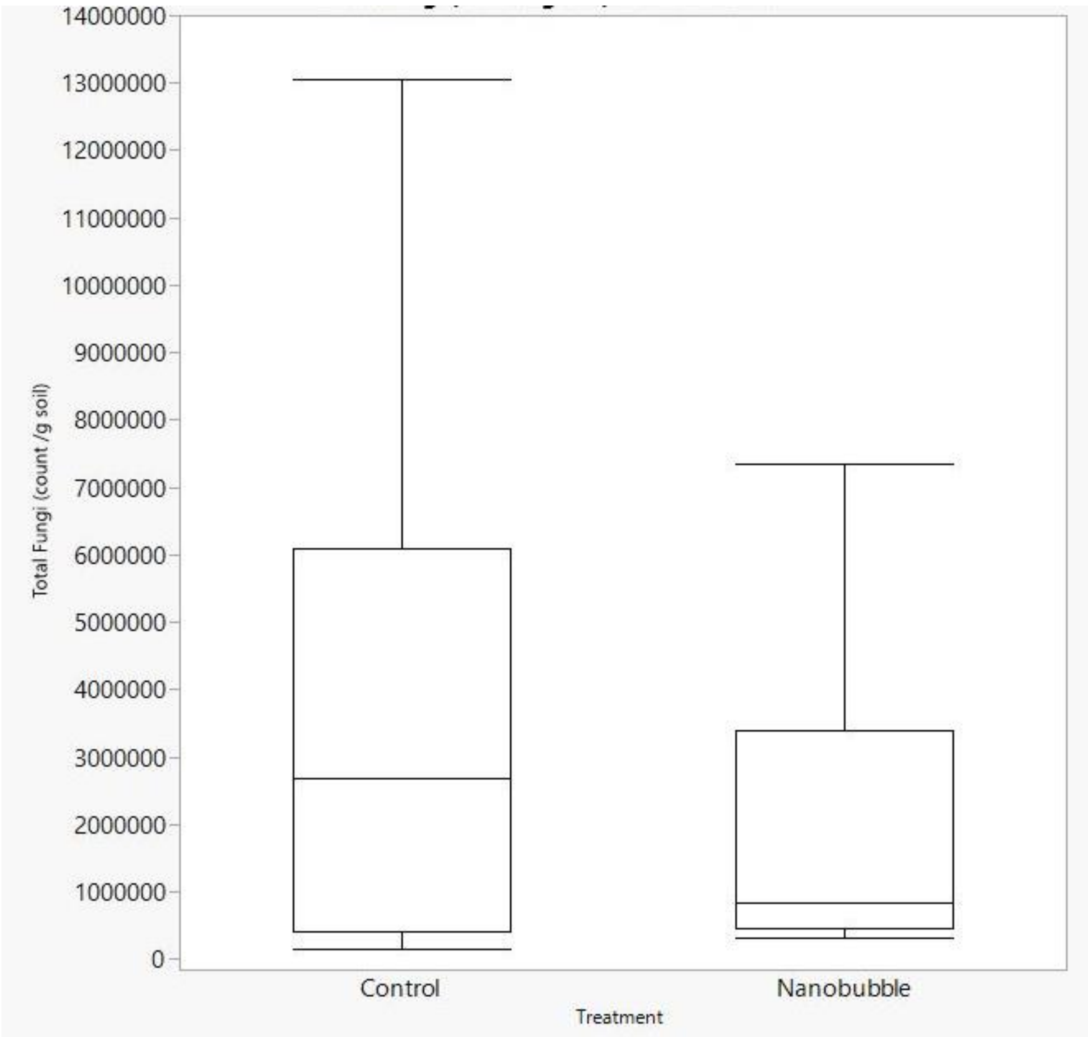

Figure 4.4: Total fungi abundance (count g^{-1} soil) in response to treatment with oxygenated nanobubble water or control pond water.

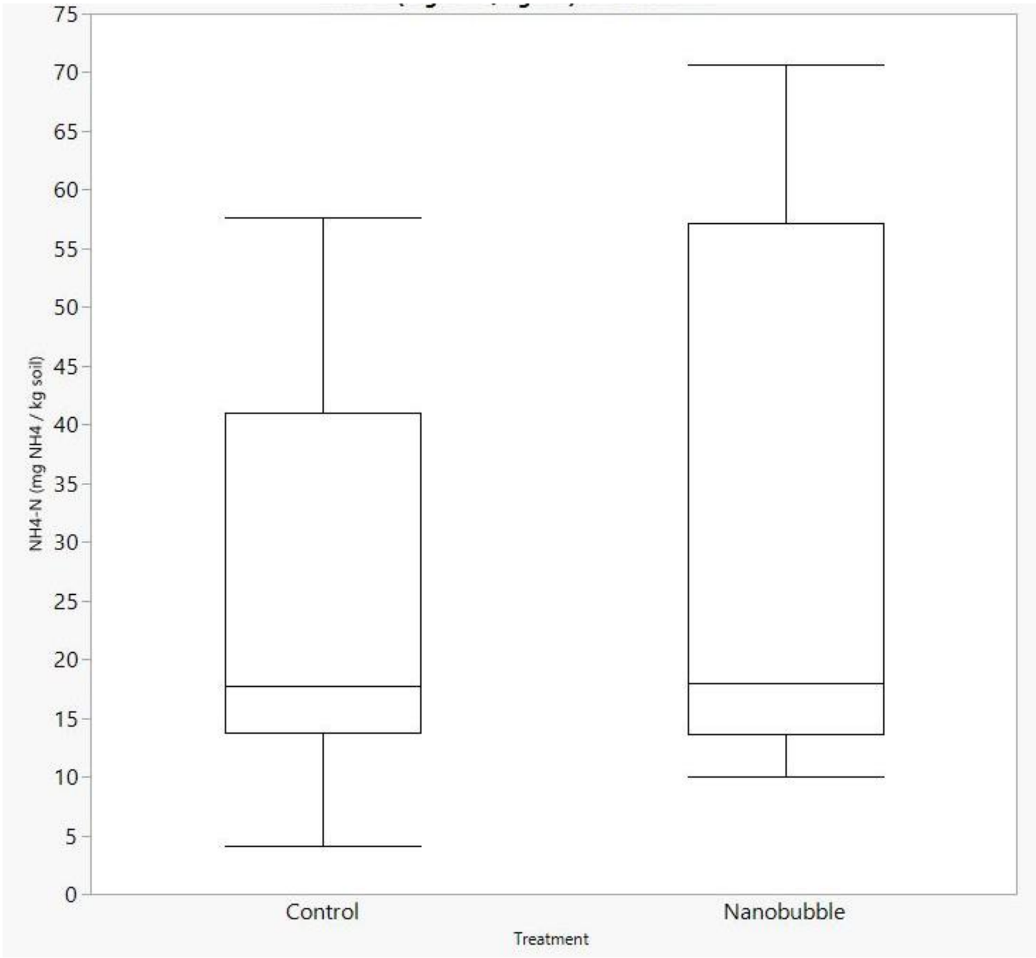

Figure 4.5: Ammonium concentration (NH_4-N) (mg NH_4 kg^{-1} soil) in response to treatment with oxygenated nanobubble water or control pond water. The treatments did not significantly affect ammonium content.

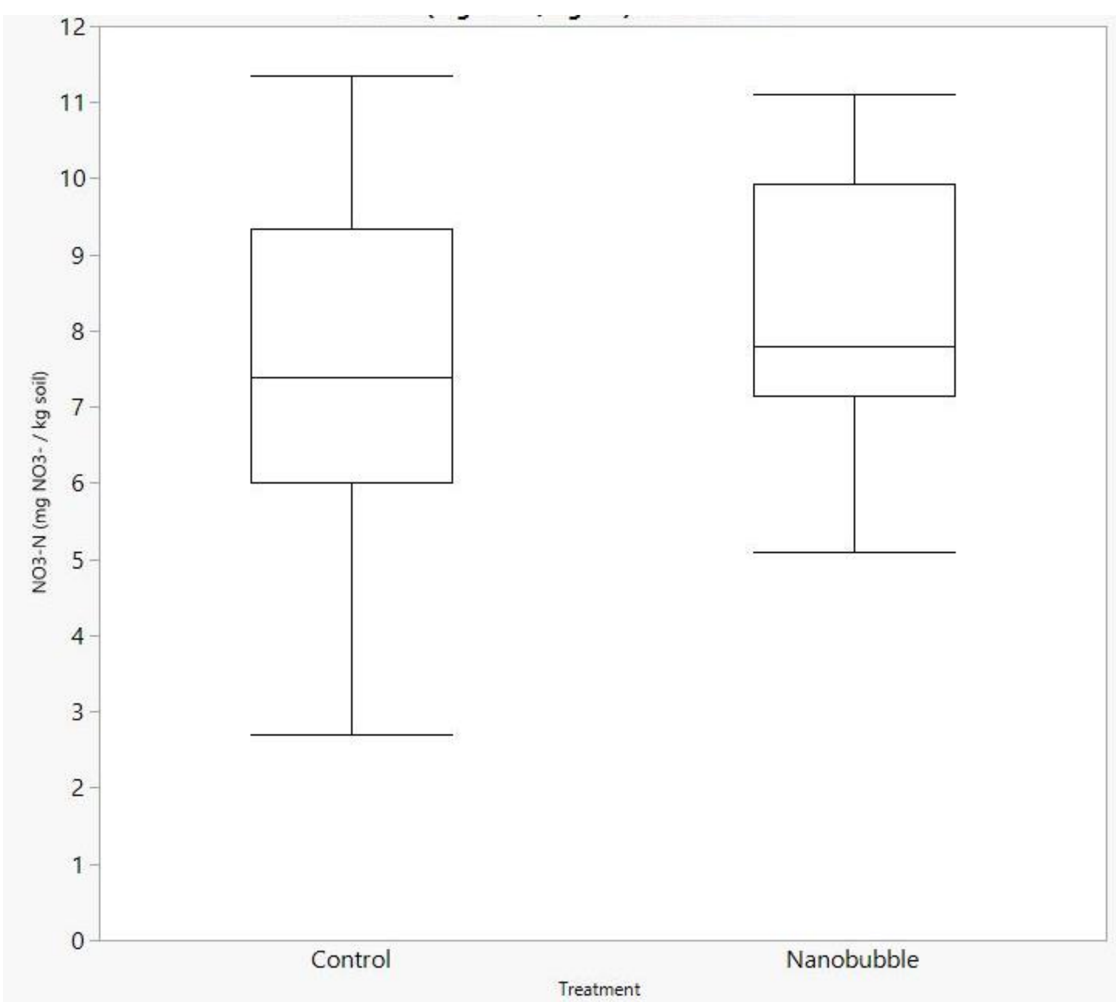

Figure 4.6: Nitrate concentration (NO_3-N) (mg NO_3^{-1} kg^{-1} soil) in response to treatment with oxygenated nanobubble water or control pond water. The treatments did not significantly affect nitrate content.

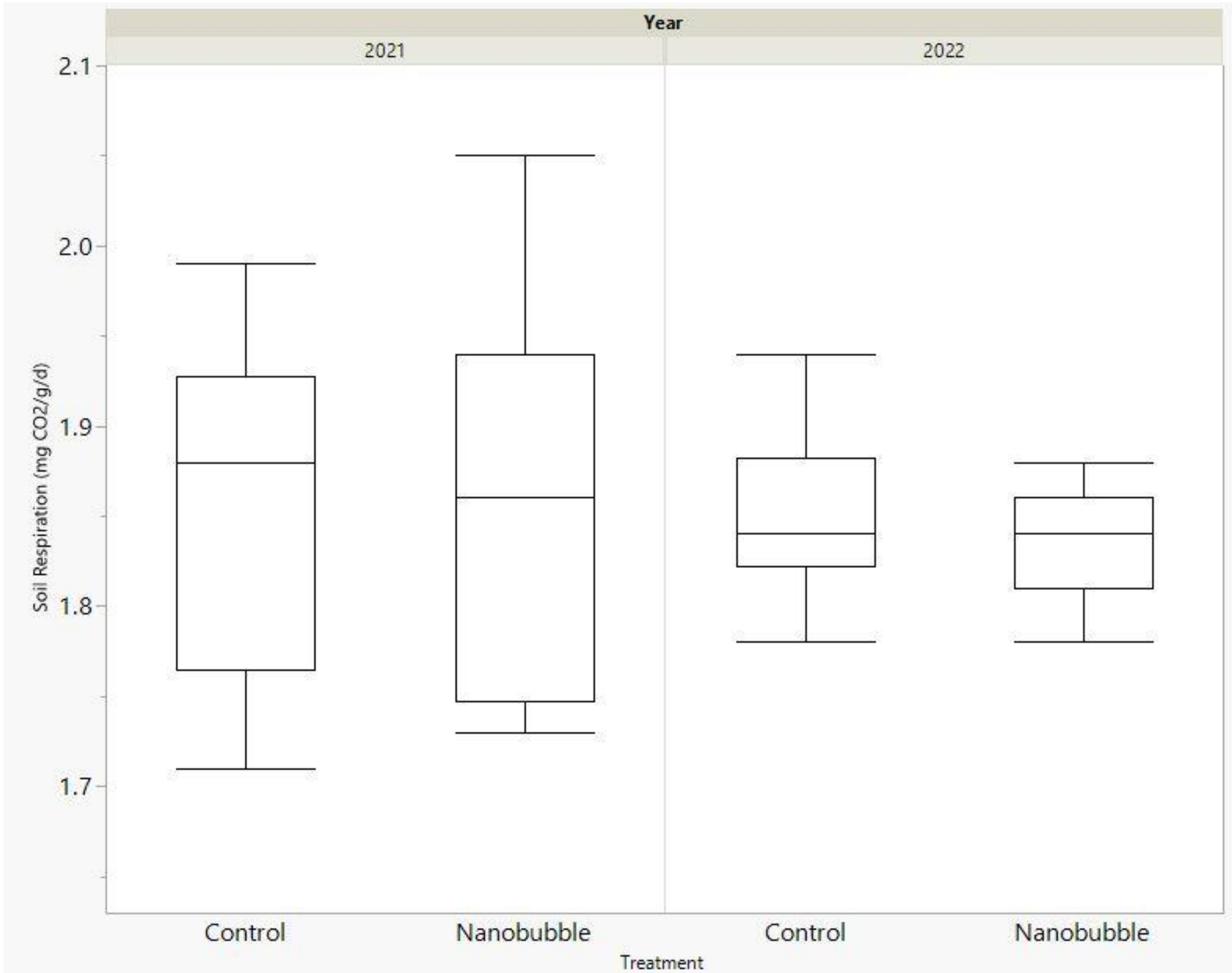

Figure 4.7: Soil respiration (mg CO_2 g^{-1} d^{-1}) in response to treatment with oxygenated nanobubble water or control pond water. The treatments did not significantly affect soil respiration.

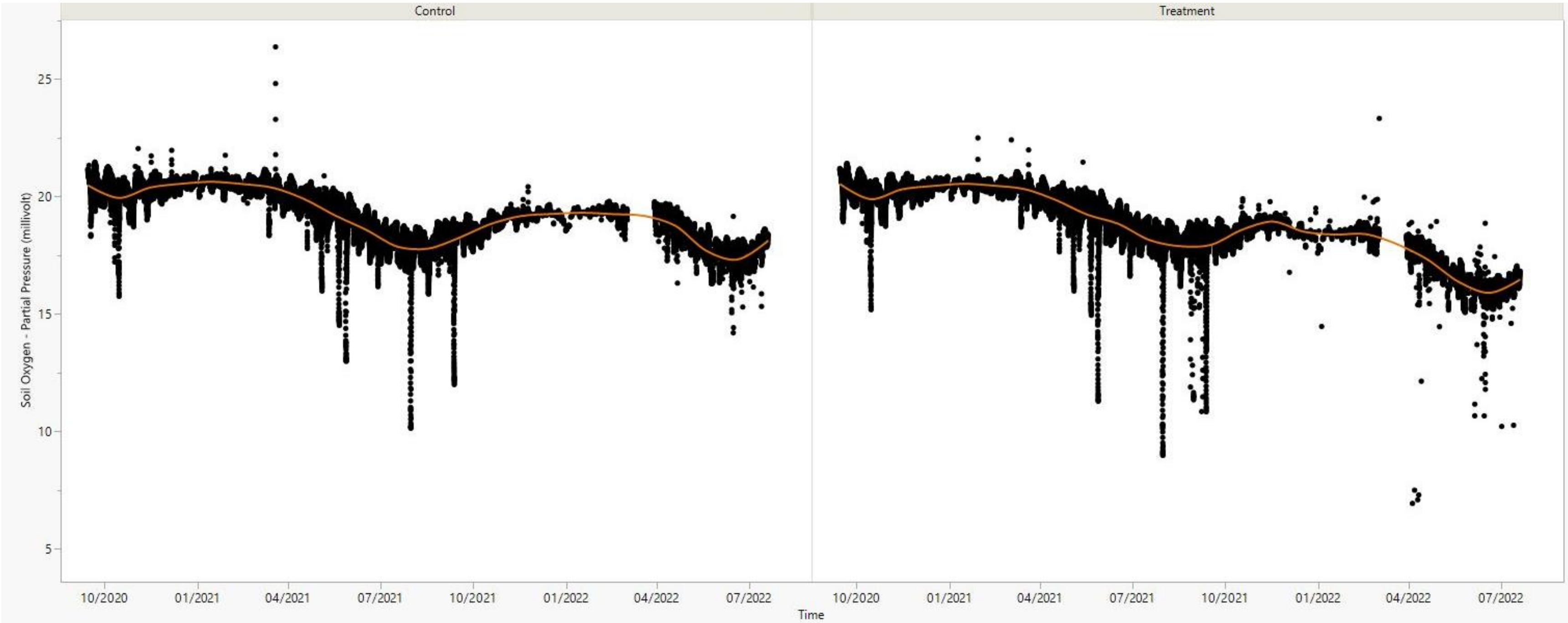

Figure 4.8: Soil oxygen (partial pressure (mV)) sensor data taken over the course of the 2-year field trials, distinguishing between control and treatment plots.

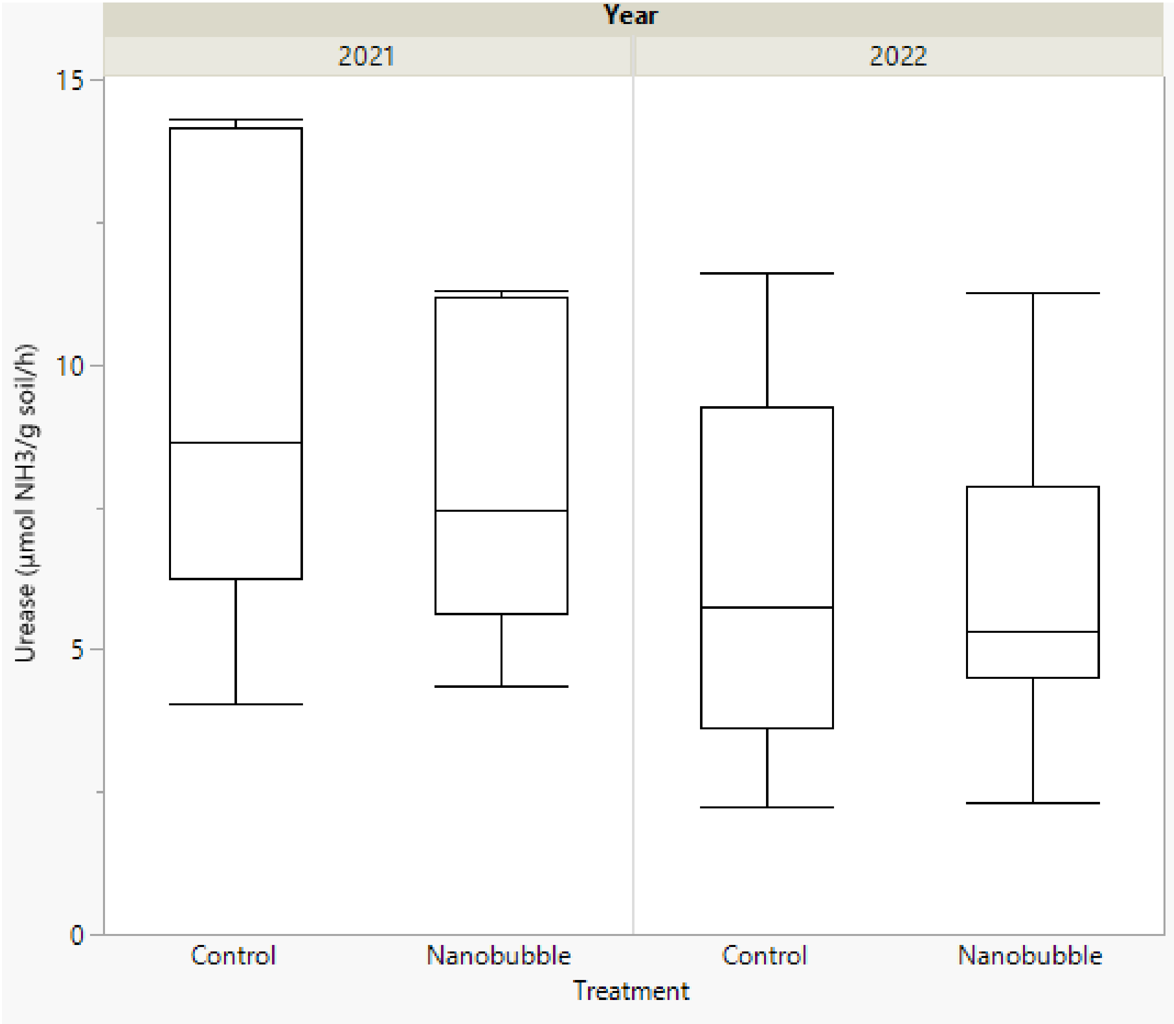

Figure 4.19: Urease activity (µmol NH_3 g^{-1} soil h^{-1}) in response to treatment with oxygenated nanobubble water or control pond water. The treatments did not significantly affect urease activity.

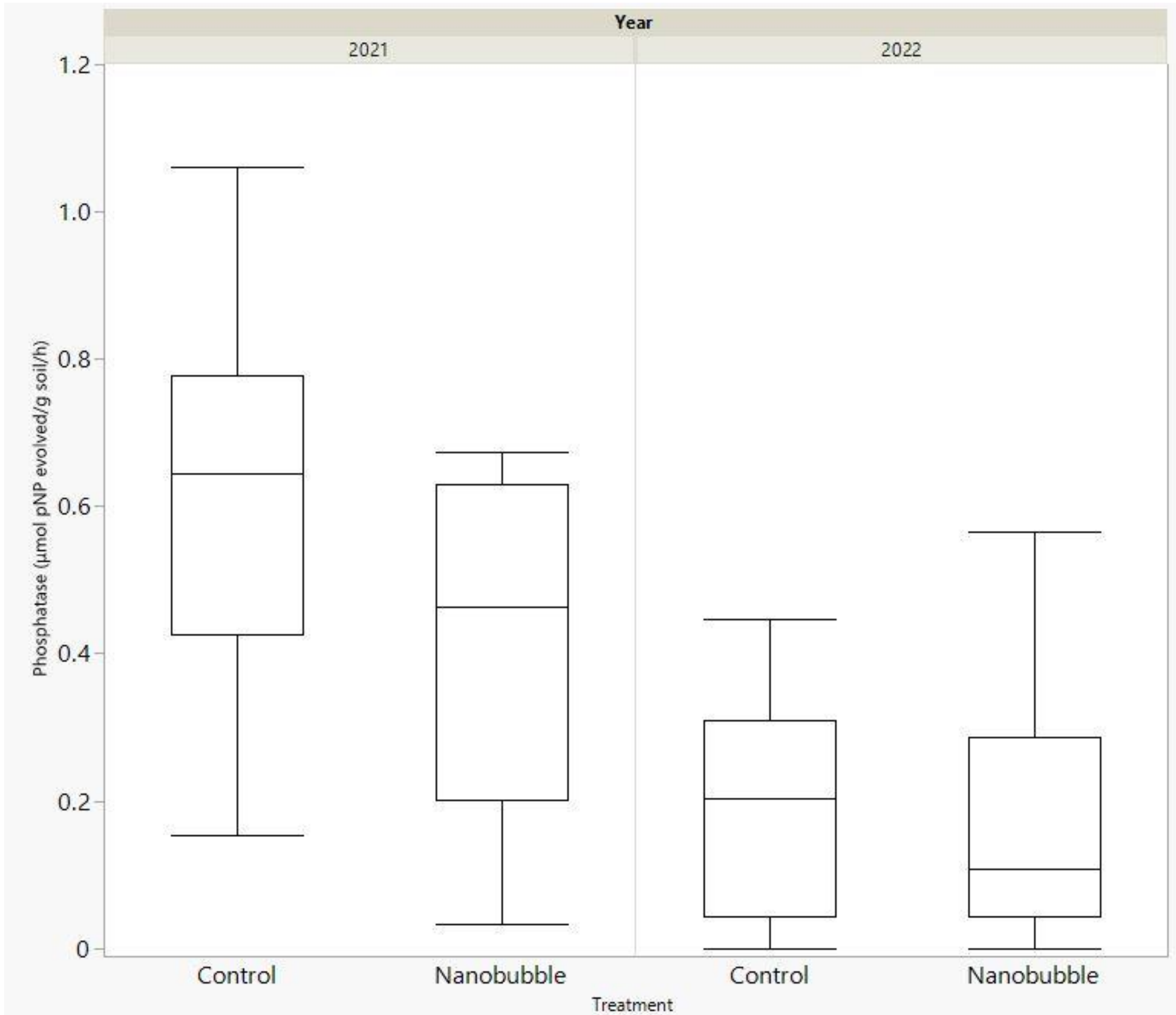

Figure 4.10: Phosphatase activity (µmol pNP g^{-1} soil h^{-1}) in response to treatment with oxygenated nanobubble water or control pond water. The treatments did not significantly affect phosphatase activity

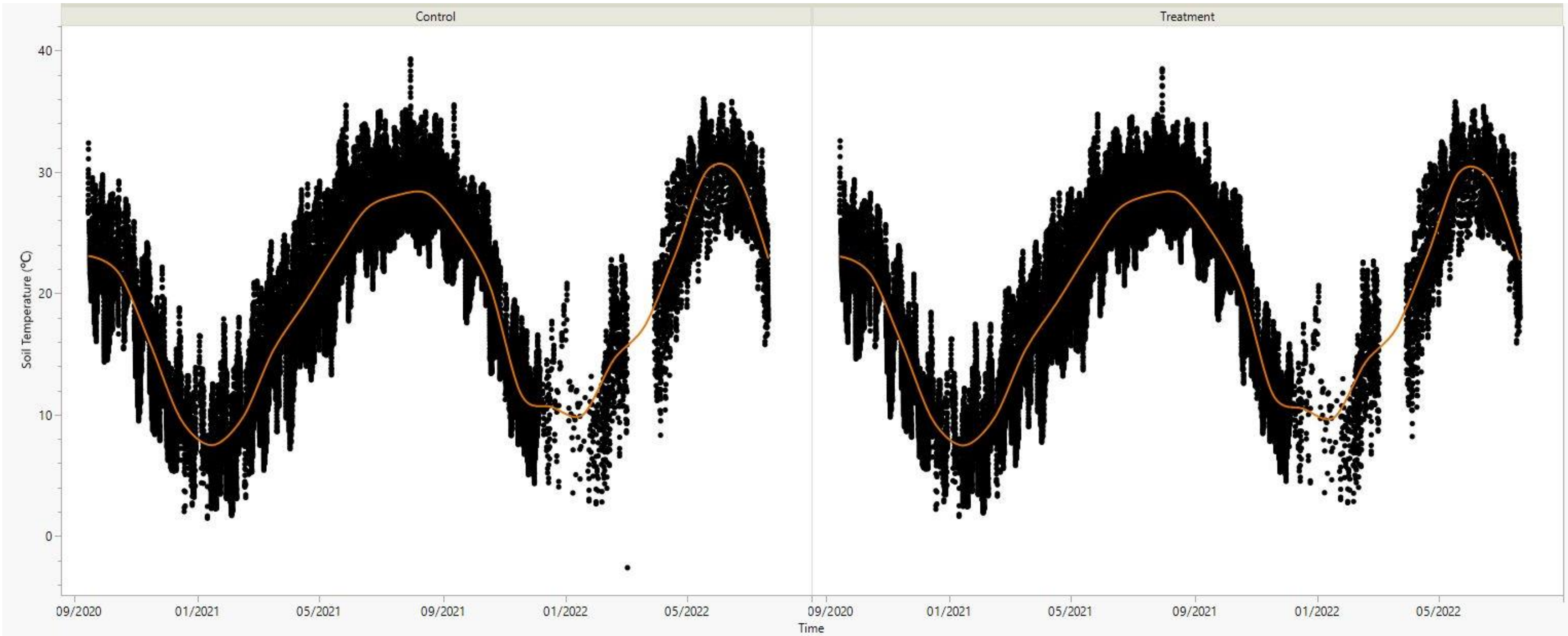

Figure 4.11: Soil temperature (°C) sensor data taken over the course of the 2-year field trials, distinguishing between control and treatment plots.

Table 4.1: Primer sequences and thermal cycling conditions used for qPCR analysis.

Target group	Gene	Amplicon length (bp)	Primers		Thermocycling conditions	Ref.
			Name	Sequence		
Total Bacteria	16S rDNA	200	Eub338	5'-ACTCCTACGGGAGGCAGCAG-3'	95ºC for 15 min; 40 cycles of 95ºC for 1 min, 53ºC for 1 min, and 72ºC for 1 min	(Fierer et al., 2005)
			Eub518	5'-ATTACCGCGGCTGCTGG-3'		
Total fungi	18s rDNA	422	Nu-SSU-0817	5'-TTAGCATGGAATAATRRAATAGGA 3'	94ºC for 10 min; 40 cycles of 94ºC for 1 min, 56ºC for 1 min, and 72ºC for 2 min	(Borneman and Hartin, 2000)
			Nu-SSU-119	5'-TCTGGACCTGGTGAGTTTCC-3'		

Table 4.2: Mixed model analysis of microbial abundance in TifEagle bermudagrass treated with nanobubble or control pond water

Response variables	Main effect *p*-value (α=0.05)
	Treatment
Total Bacteria (counts g^{-1} soil)	0.4472
Total Fungi (counts g^{-1} soil)	0.2223

Table 4.3: Mixed model analysis of Nitrate (NO_3-N) and Ammonium (NH_4-N) content in TifEagle bermudagrass treated with nanobubble or control pond water

Response variables	Main effect *p*-value (α=0.05)
	Treatment
Nitrate (NO_3-N) (mg NO_3^{-1} kg^{-1} soil)	0.2793
Ammonium (NH_4-N) (mg NH_4 kg^{-1} soil)	0.3044

Table 4.4: Mixed model analysis of enzyme activities and soil respiration in TifEagle bermudagrass treated with nanobubble or control pond water.

Response variables	Main effect *p*-value (α=0.05)					
	2021			2022		
	Sampling Date	Treatment	SD-T	Sampling Date	Treatment	SD-T
Soil respiration (mg CO_2 g^{-1} soil d^{-1})	<0.0001	0.1889	0.0273	0.0225	0.1211	<0.0001
Urease activity (μmol NH_3 g^{-1} soil h^{-1})	<0.0001	0.1254	0.9053	0.0006	0.6725	0.0015
Phosphatase activity (μmol pNP g^{-1} soil h^{-1})	0.2184	0.4110	0.6724	0.0028	0.9599	0.9757

Table 4.5: ANOVA table for soil oxygen (mV) and soil temperature (°C) from Apogee SO-110 Soil Response Thermistor Reference Oxygen Sensors (Apogee Instruments, Logan, UT).

Response parameters	Main effect *p*-value (α=0.05)		
	Time	Treatment	Time*Treatment
Soil Oxygen	1.000	<0.0001	1.000
Soil Temperature	1.000	0.3217	1.000

Table 4.6: Soil oxygen and soil temperature measurements in response to treatment with oxygenated nanobubble water and treatment of control pond water. Means with same letter suffixes are not significantly different from each other.

RP	Treatment	
Soil Oxygen Partial Pressure (millivolt)	Nanobubble Water	19.01 b
	Control	19.24 a
Soil Temperature (°C)	Nanobubble Water	19.96 a
	Control	19.90 a

Table 4.7: Dissolved oxygen(mg/L) and temperature (°C) taken during the field trials showing the loss in dissolved oxygen when transferring oxygenated nanobubble water from container to spray nozzle for treatment application.

Dissolve oxygen of pond water in a tank	Dissolved oxygen of pond water at the nozzle	Dissolved oxygen of oxygenated nanobubble water in the mix tank	Dissolved oxygen of oxygenated nanobubble water at the nozzle	Temperature
5.19 mg L^{-1}	6.24 mg L^{-1}	31.62 mg L^{-1}	22.11 mg L^{-1}	17.70°C

Table 4.8: ANOVA table for shoot weight, root weight, digital imagery for turf quality, and visual rating for turf quality

Main effect *p*-value (α=0.05)						
Response parameters	2021			2022		
	Sampling Date	Treatment	SD-T	Sampling Date	Treatment	SD-T
Shoot Weight	<0.0001	0.5083	<0.0001	0.1231	0.4728	0.9923
Root Weight	0.0003	0.5174	0.2500	0.0051	0.0468	0.7279
Digital Imagery	<0.0001	0.8353	0.9142	<0.0001	0.4064	0.2263
Visual Rating	<0.0001	0.6579	0.3312	<0.0001	1.0000	1.0000

Table 4.9: Mean digital turf quality (% green cover) and visual turf quality (1-9 scale) in response to treatment with oxygenated nanobubble water and treatment of control pond water. Means with the same letter suffixes are not significantly different from each other.

RP	Treatment	2021	2022
Digital Imagery (% area greenness)	Nanobubble Water	84.9 a	88.4 a
	Control	84.7 a	89.4 a
Visual Rating (1-9)	Nanobubble Water	8.77 a	8.32 a
	Control	8.75 a	8.32 a

Table 4.10: Mean plant shoot weight (g) and root weight (g) in response to treatment with oxygenated nanobubble water and treatment of control pond water. This means with the same letter suffixes are not significantly different from each other.

RP	Treatment	2021	2022
Grass Weight (g)	Nanobubble Water	1.76 a	2.81 a
	Control	1.66 a	2.97 a
Root Weight (g)	Nanobubble Water	0.090 a	0.135 a
	Control	0.097 a	0.107 b

Table 4.11: ANOVA table for root growth traits

Response parameters	Main effect *p*-value (α=0.05)					
	2021			2022		
	Sampling Date	Treatment	SD-T	Sampling Date	Treatment	SD-T
Average Root Width	<0.0001	0.0357	0.1341	0.1897	0.1533	0.9372
Max. Number of Roots	0.0261	0.9201	0.4634	<0.0001	0.1011	0.0441
Med. Number of Roots	0.0158	0.8125	0.5066	<0.0001	0.2830	0.1642
Number of Connected Components	<0.0001	0.6564	0.6786	0.4230	0.3741	0.1658

Table 4.12: Mean root growth traits in response to treatment with oxygenated nanobubble water and treatment of control pond water. Means with the same letter suffixes are not significantly different from each other.

RP	Treatment	2021	2022
Average Root Diameter (cm)	Nanobubble Water	0.022 a	0.027 a
	Control	0.021 b	0.026 a
Max. Number of Roots (n)	Nanobubble Water	31.9 a	28.6 a
	Control	31.6 a	25.5 a
Median Number of Roots (n)	Nanobubble Water	22.7 a	20.3 a
	Control	23.3 a	18.6 a
Number of Connected Components (n)	Nanobubble Water	64.9 a	102.0 a
	Control	62.1 a	94.5 a

CHAPTER 5

SUMMARY and CONCLUSIONS

In this study, the impacts of oxygenated nanobubble water on turf growth and quality, soil biological health, and water movement and retention were evaluated in the greenhouse and field. In the greenhouse study, the impact of the nanobubble water was evaluated at two levels of irrigation, and measurements of turf growth and quality, water movement and retention, and soil biological health were made over time after multiple applications. It was found that there were significant treatment effects on the infiltration rate as the control exhibited a faster infiltration rate compared to that of the oxygenated nanobubble treatment pots. This does not align with previous research as it has been reported that the use of oxygenated nanobubble water may alter soil surface charges and reduce their hydrophobic bonds.

There were no significant treatment effects on many of the turf growth parameters indicated by the root scanning analysis. When there were treatment effects, (e.g., average root width, the median number of roots, and root length), they were inconsistent between the years. There was a significant effect of oxygenated nanobubble water on maximum root length in 2022 but was not replicated in 2021. Maximum root length in 2022 showed that the control root length was longer than the nanobubble root length. There was no effect of oxygenated nanobubble water on root weight or shoot weight. Thatch weight showed an effect of the treatment in 2021, with the control thatch weight being higher than the nanobubble water-treated thatch weight, but this was not seen again in the 2022 greenhouse trial.

There was a treatment effect on clipping weight, with a higher clipping weight for oxygenated nanobubble water than control in 2021, but a higher clipping weight for control than nanobubble water treatment in 2022. Relative leaf water content was affected by oxygenated nanobubble water in 2021, with the control having a higher % relative leaf water content than the nanobubble treatment. This was not seen again in 2022. Turf quality was significantly affected by oxygenated nanobubble water in 2021, showing higher % area greenness in the control than in the nanobubble treatment, but the pattern switched in 2022 with nanobubble treatment yielding higher % area greenness. The visual rating showed a higher effect on nanobubble water in 2021, with a higher score for nanobubble water but showed no effect in 2022 with no significance of the treatment.

For the field study, experimental plots were established in a golf course green and the impact of oxygenated nanobubble water on turf growth and quality, and soil biological health and abundance were evaluated over time after multiple applications during the summer growing season. Soil biological health was assessed by measuring parameters that included microbial abundance (total bacteria and total fungi), inorganic nitrogen content (nitrate and ammonium), and activity (soil respiration and enzyme activities).

Treatment in the field with oxygenated nanobubble water did not significantly impact soil respiration, urease enzyme activity, or phosphatase enzyme activity in the field trials. There were no significant treatment effects on inorganic nitrogen content or microbial abundance as well. Neither was there any significant effect of oxygenated nanobubble water on root trait parameters, shoot weight and turf quality. There were significant treatment effects in 2022 for root weight, as oxygenated nanobubble treatment yielded a higher root weight than the control, but this was not replicated in 2021. Average root width did show significance from the digital root scanning in 2021 but was small in difference even with its significant treatment effects. It is also relevant to the field trial in that there were significant treatment effects for soil oxygen, with the control showing a higher mean soil oxygen concentration than that of the oxygenated nanobubble treatment.

Overall, the study is an attempt in providing some insight into the effect of oxygenated nanobubble water on turfgrass growth and quality, water movement and retention, and soil biological health and abundance. Some of the inconsistencies found in the data may be attributed to the loss of oxygenated nanobubbles from the time of generation in the tank to the time of application out in the greenhouse or field, which may have been attributed to the lack of significant treatment effects. In addition, as indicated by the soil oxygen sensors in the field trial since August 2020, oxygenated nanobubbles were not staying with the soil or had a short residence time. This fact may also have attributed to the lack of significant treatment effects. The lack of treatment effect might also be due to oxygen not being a limiting factor. Additionally, there may have been external factors that further affected the response parameters measured in greenhouse and field studies.

Based on these limited studies, the data suggest that the technology did not benefit the turfgrass system as expected. Many problems need to be addressed before marketing this technology for use in a golf courses turfgrass system, such as the loss of dissolved oxygen from time of generation to time of application and the short residence time of the oxygenated nanobubbles in the soil. Further evaluations of oxygenated nanobubble technology are needed in the applications of a turfgrass system, especially under varying gradients of oxygen availability to see if the oxygenated nanobubbles affect the turf differently under oxygen-stressed environments.

APPENDIX A

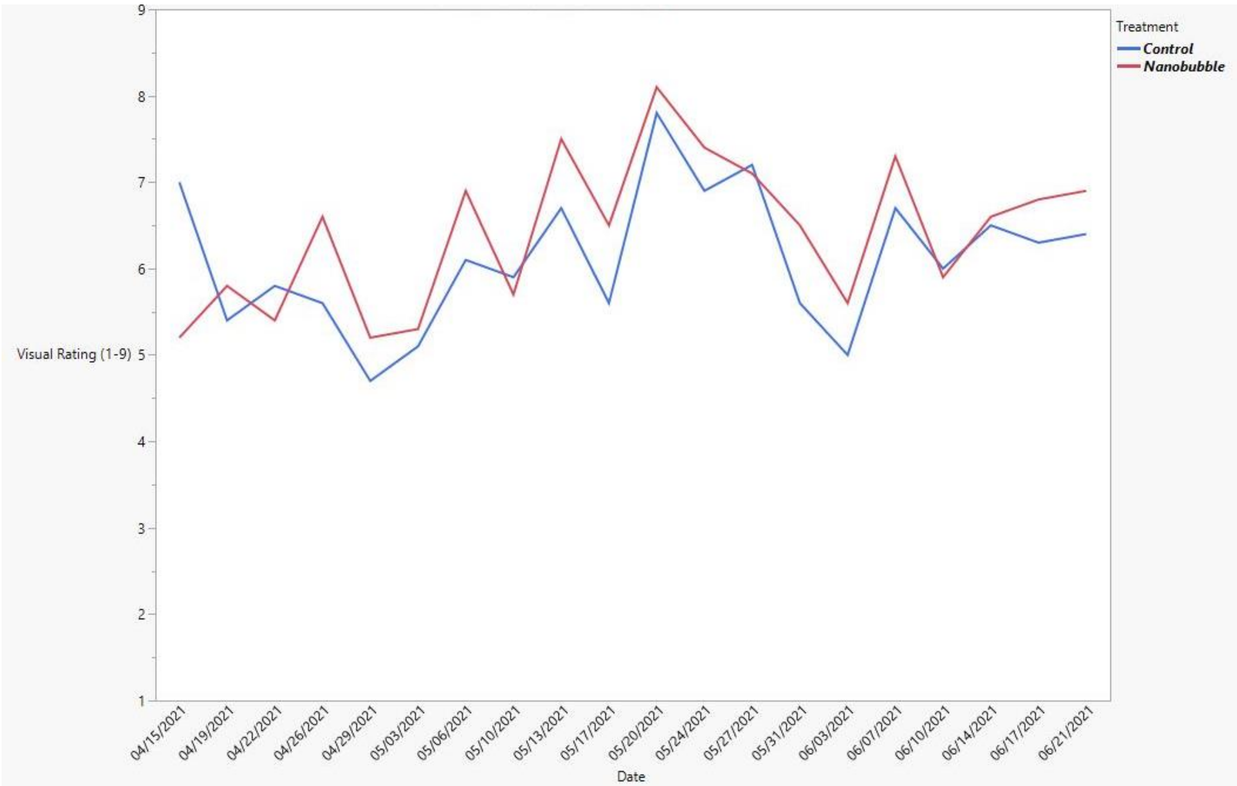

Figure A.1: Visual Rating (1-9) taken over the course of the 2021-year greenhouse trials, distinguishing between control and treatment.

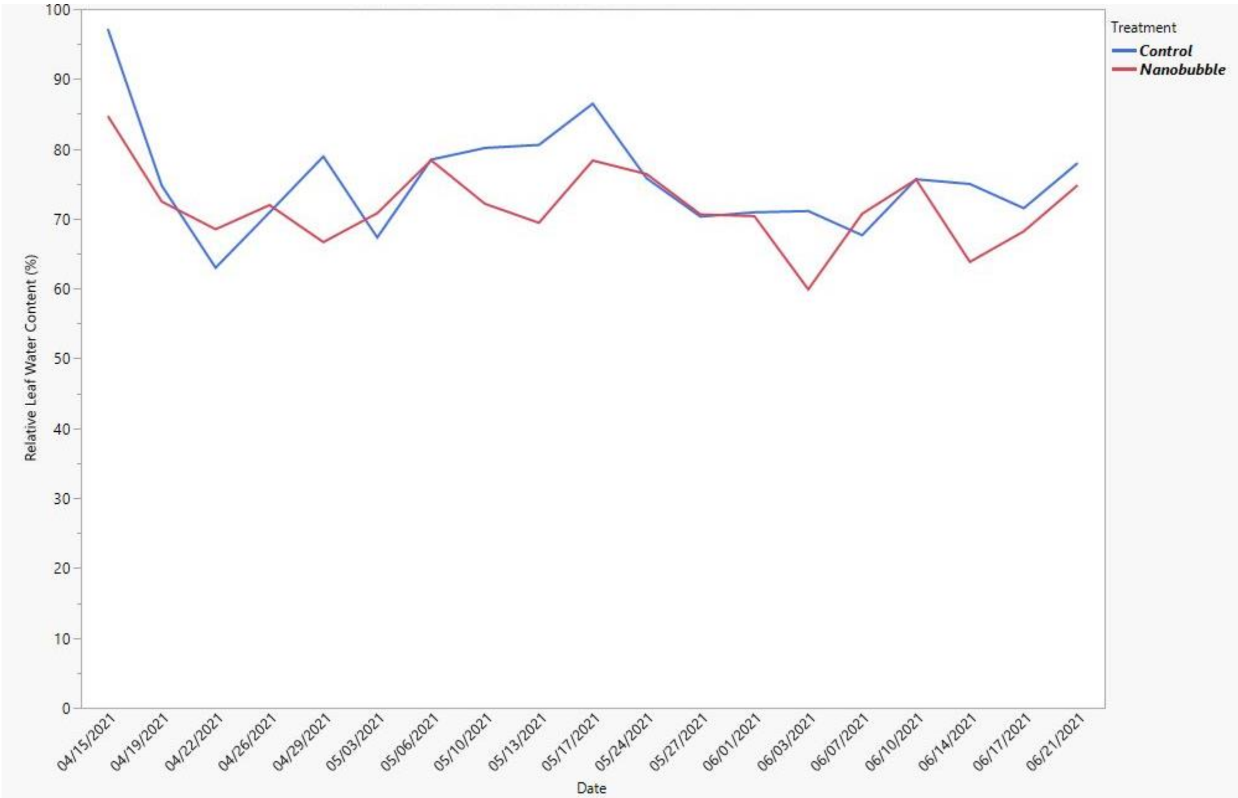

Figure A.2: Relative leaf water content taken over the course of the 2021-year greenhouse trials, distinguishing between control and treatment.

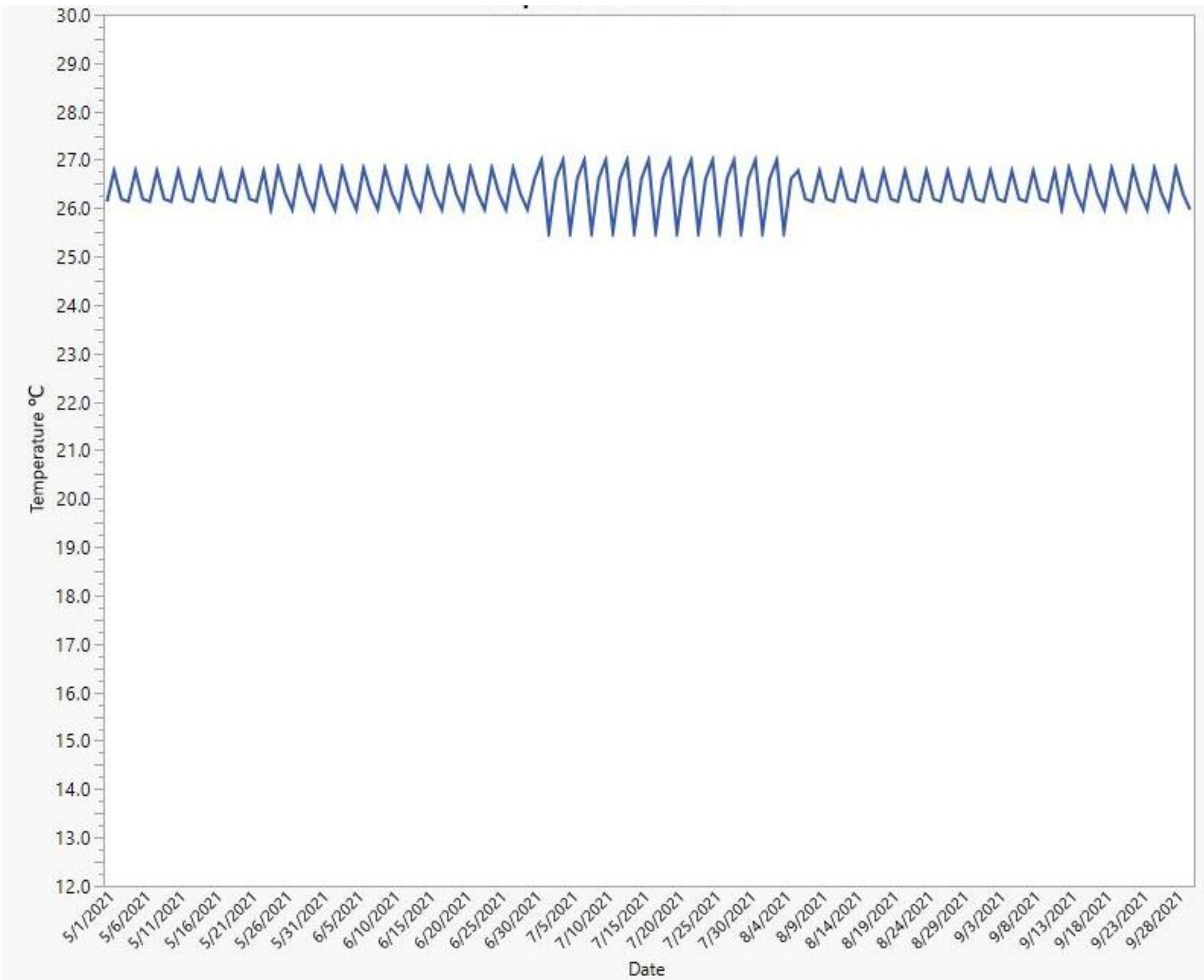

Figure A.3: Temperature (°C) taken over the course of the 2021-year field trials. Data was pulled from the University of Georgia weather network from Johns Creek, Fulton County, Georgia.

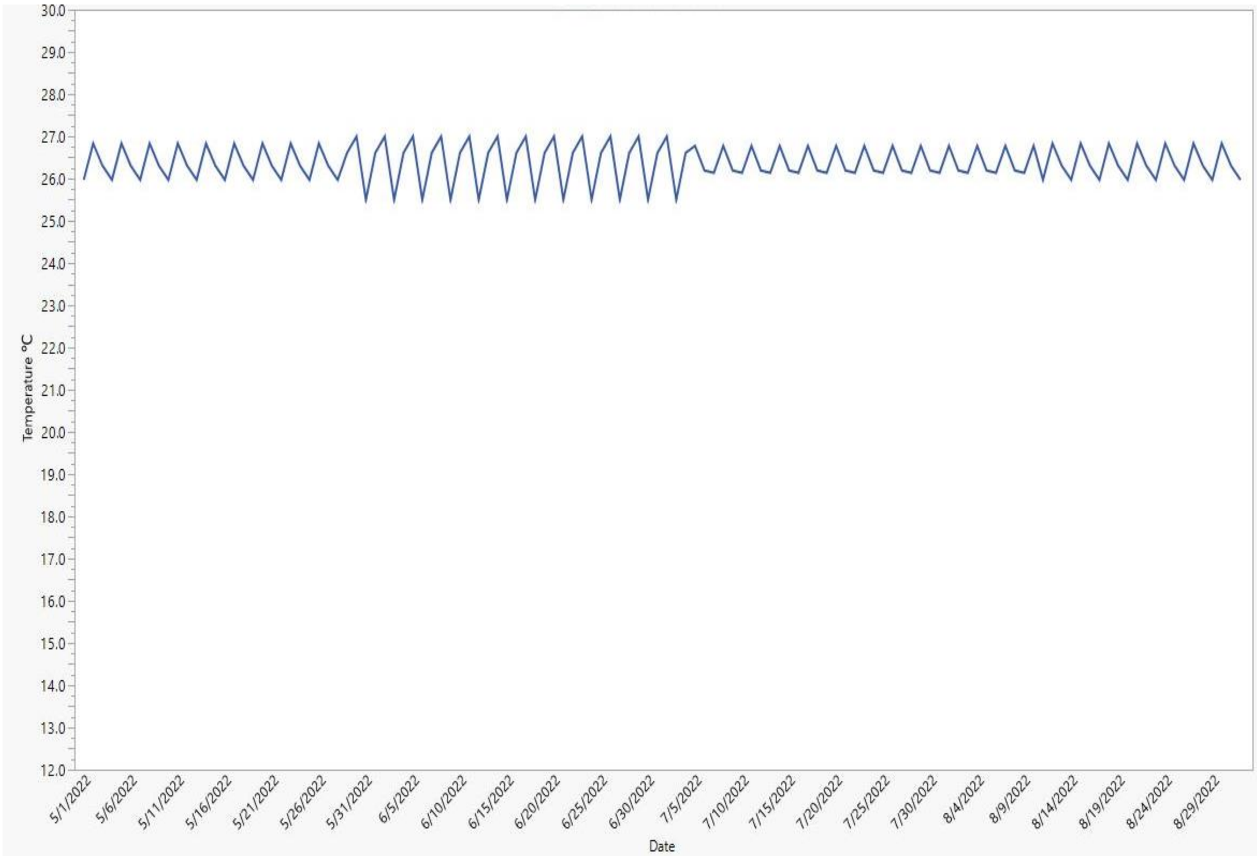

Figure A.4: Temperature (℃) taken over the course of the 2022-year field trials. Data was pulled from the University of Georgia weather network from Johns Creek, Fulton County, Georgia.

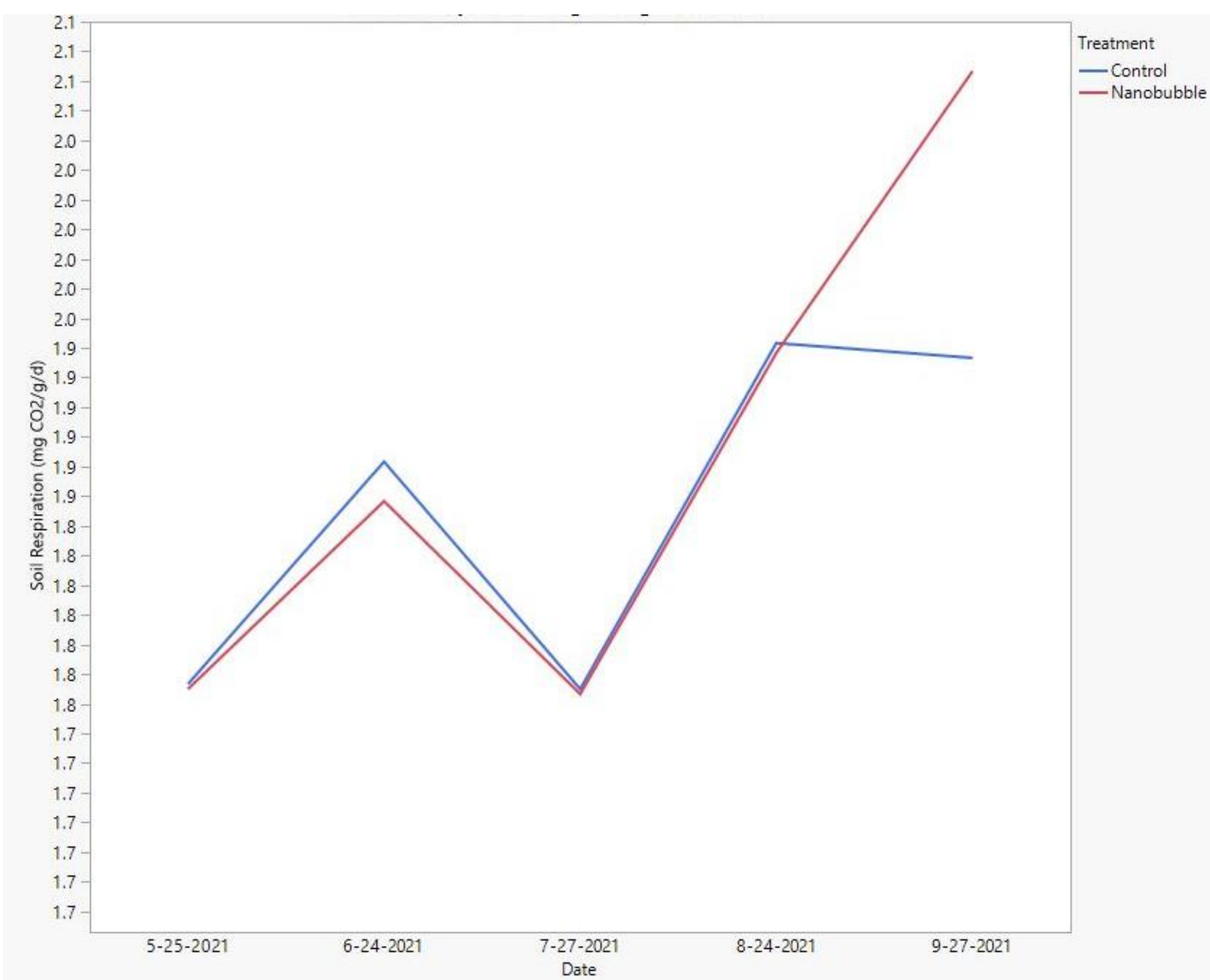

Figure A.5: Soil respiration (mg CO_2/ g/ d) taken over the course of the 2021-year field trials, distinguishing between control and treatment plots.

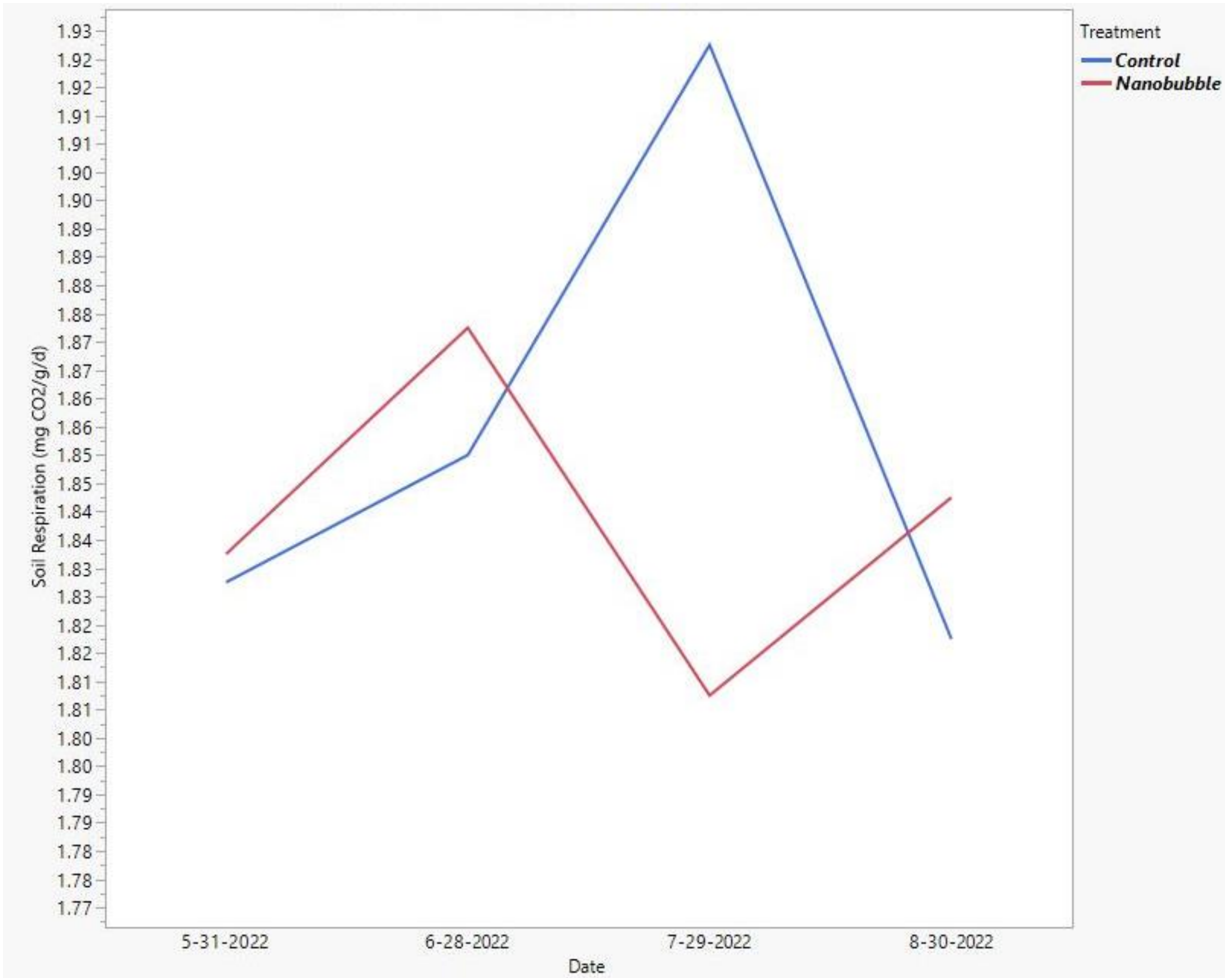

Figure A.6: Soil respiration (mg CO_2/ g/ d) taken over the course of the 2022-year field trials, distinguishing between control and treatment plots.

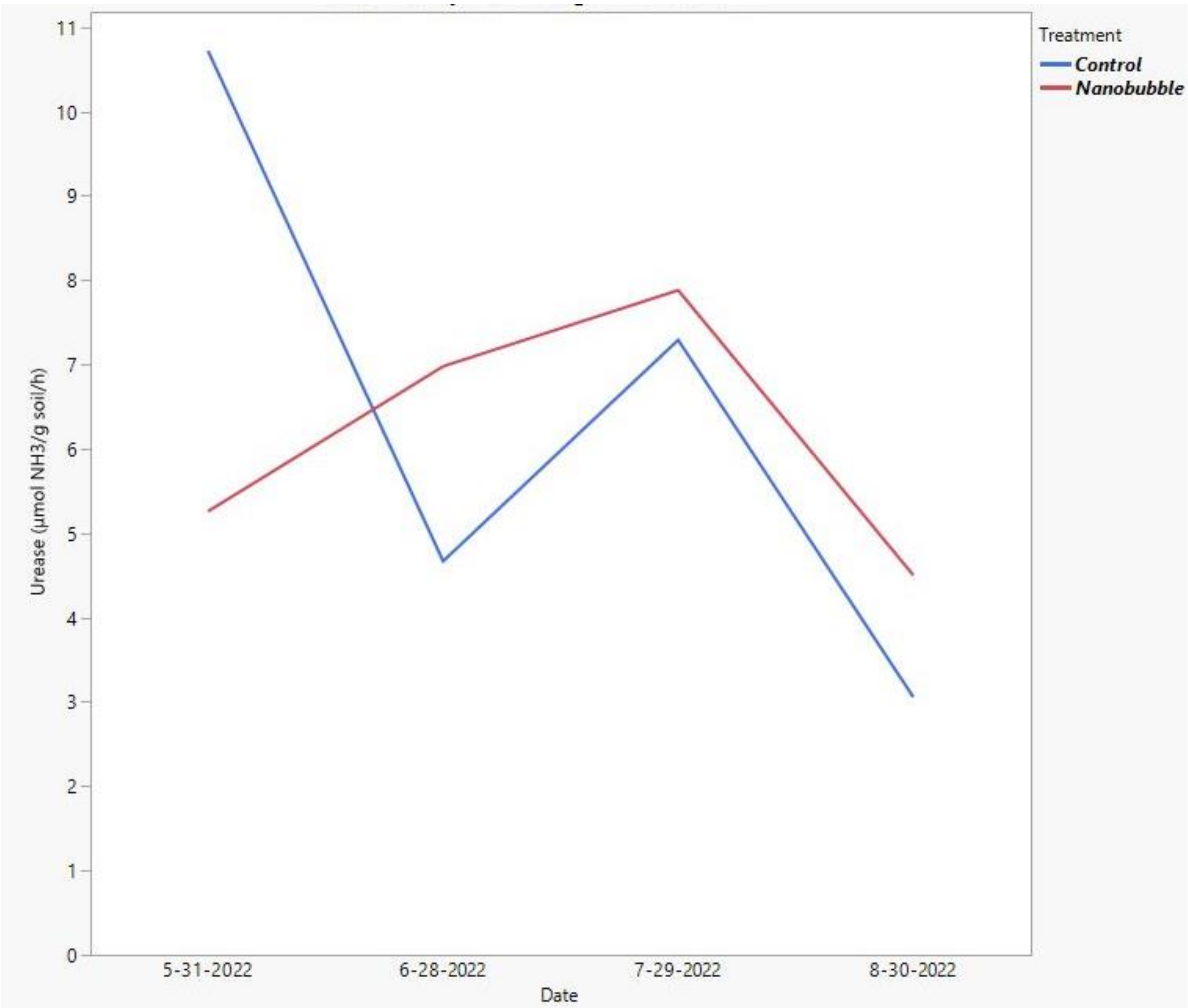

Figure A.7: Urease activity (µmol NH_3 g^{-1} soil h^{-1}) taken over the course of the 2022-year field trials, distinguishing between control and treatment plots.

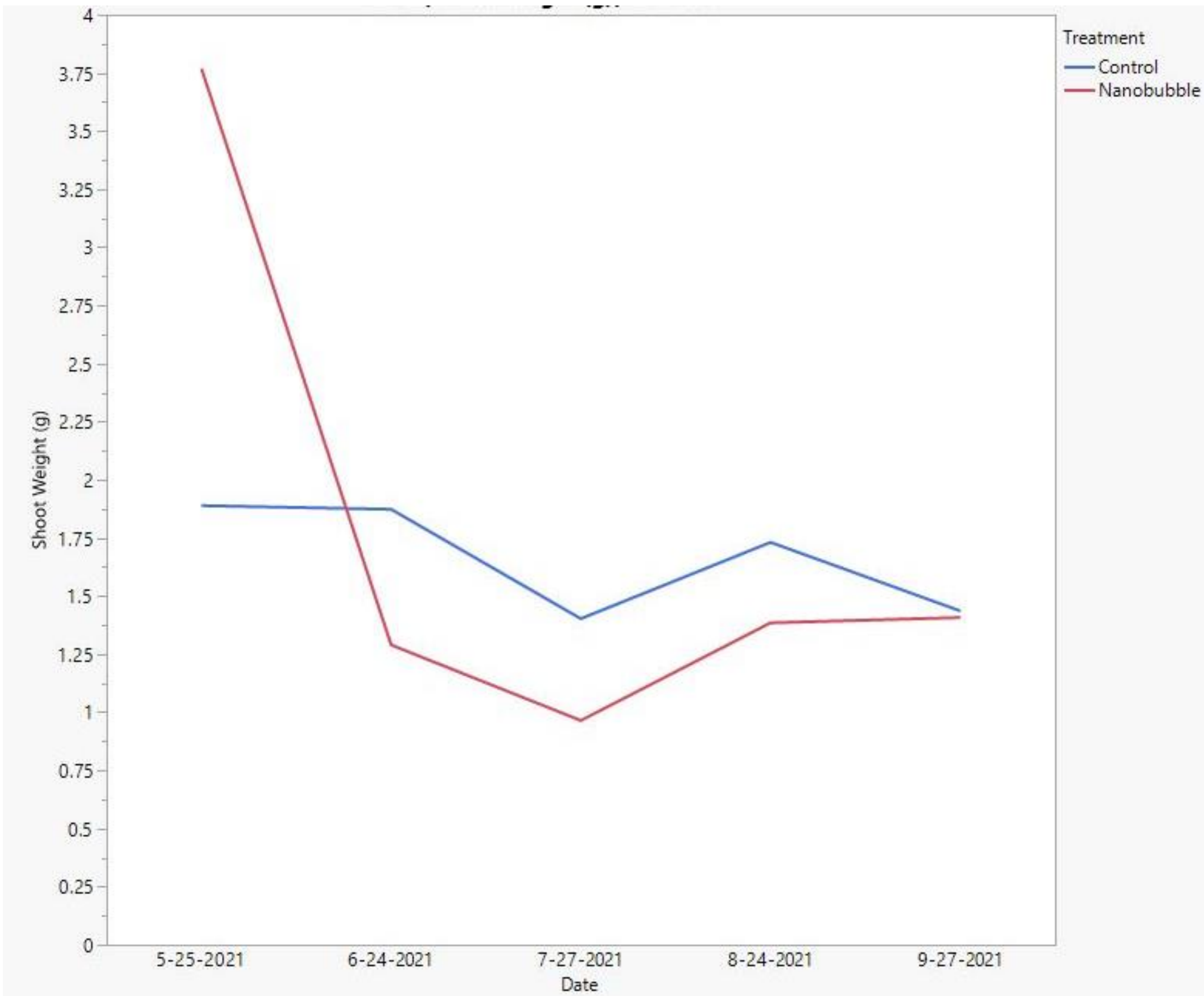

Figure A.8: Shoot weight (g) taken over the course of the 2021-year field trials, distinguishing between control and treatment plots.

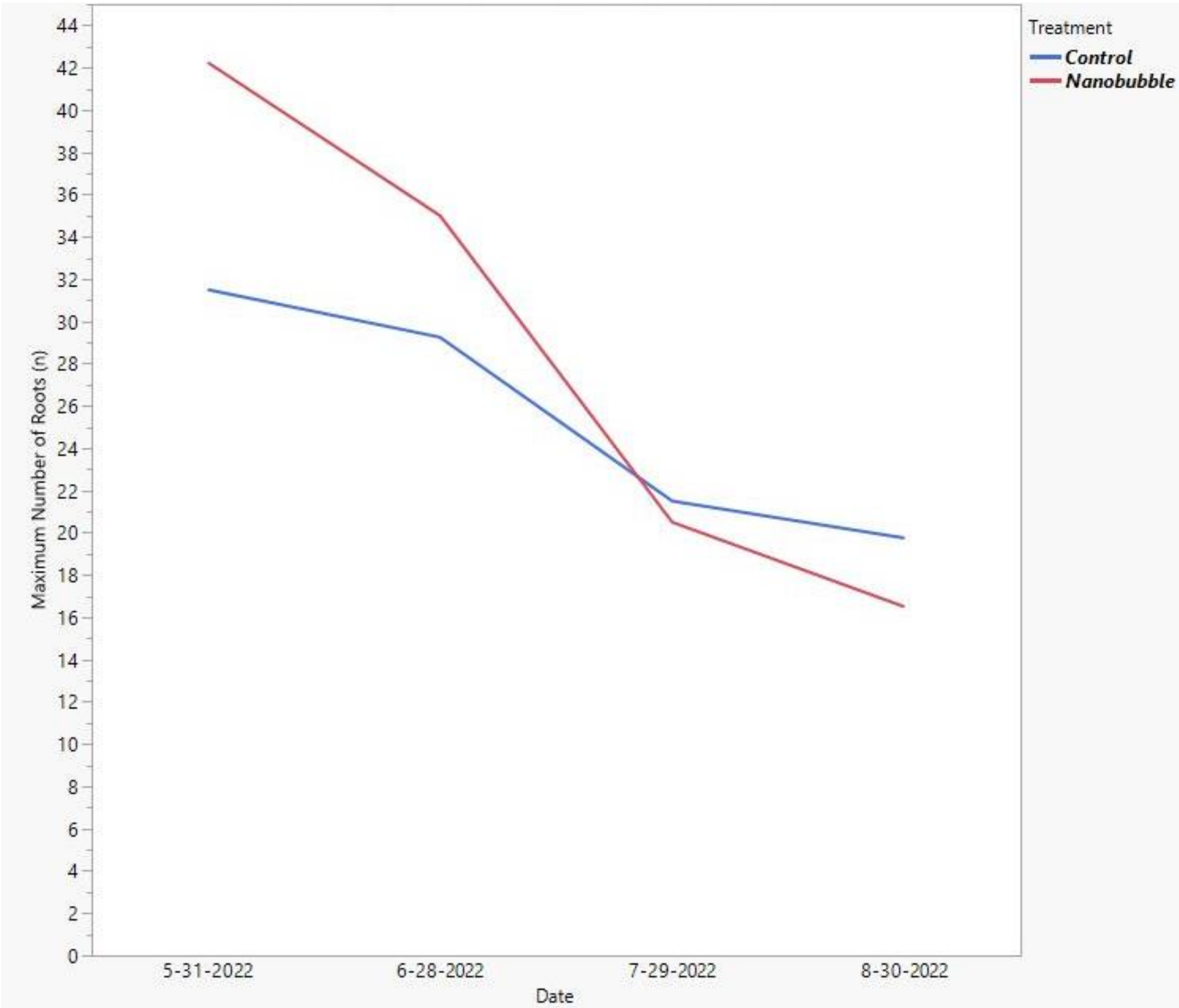

Figure A9: Maximum number of roots taken over the course of the 2022-year field trials, distinguishing between control and treatment plots.