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Effect of Nanobubble-Oxygenated Water for Irrigation of Sand-Based Golf Course Putting Greens

Eric DeBoer
University of Arkansas, Fayetteville

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Irrigation of Sand-Based Golf Course Putting Greens

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by

Eric DeBoer
Michigan State University
Bachelor of Science in Crop and Soil Science, 2010
University of Arkansas
Master of Science in Horticulture, 2018

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University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

Michael Richardson, Ph.D.
Dissertation Director

Douglas Karcher, Ph.D.
Ex-Officio Member

Ryan Dickson, Ph.D.
Committee Member

Brian Haggard, Ph.D.
Committee Member

William Berndt, Ph.D.
Ex-Officio Member

Abstract

Nanobubbles (NBs) are sub-micron, stable cavities of gas. The properties of NBs theoretically allow long bubble residence times and high gas dissolution rates resulting in the supersaturation of oxygen in water. Adequate soil aeration is a constant concern for producers of agricultural and horticultural crops. Because of their unique properties, NBs have been investigated for oxygenating water used in the irrigation of various agricultural and horticultural crops to increase soil oxygen concentration. Creeping bentgrass (*Agrostis stolonifera* L.) is the most widely used turfgrass for golf course putting green surfaces. Golf course superintendents use various methods to increase soil oxygen in putting green root zones, such as core aerification, solid-tine aerification, and sand topdressing. An alternative method of delivering oxygen to the soil could be through irrigation with NB-oxygenated irrigation water (NOIW). The objectives of this research were to 1) Examine the effect of time and temperature on the dissolved oxygen content of nanobubble-oxygenated irrigation water sources, 2) examine the effects of nanobubble-oxygenated irrigation water on infiltration, soil oxygen concentration, soil organic matter, and plant performance of a creeping bentgrass putting green, and 3) examine the effects of subsurface irrigation with nanobubble-oxygenated irrigation water on plant performance of creeping bentgrass grown in sand-based lysimeters. Four treatments included potable water, irrigation pond water, and potable and irrigation pond water oxygenated using a membrane-based NB injection system. Nanobubble oxygenation of water failed to increase the retention time of dissolved oxygen compared to a standard oxygenation method, regardless of water temperature. Nanobubble-oxygenated water did not affect creeping bentgrass plant performance in either field or laboratory settings. Nanobubble-oxygenated water increased soil oxygen in one season, but this effect was not repeatable. The organic matter content of a sand-based creeping bentgrass putting green was unaffected by irrigation with NB-oxygenated water.

Nanobubble oxygenation remains an excellent means of efficiently oxygenating large volumes of water, although plant responses to the water were negligible. Future research investigating the irrigation of other agricultural and horticultural crops is warranted as the understanding and depth of knowledge surrounding NB technology continue to improve.

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Table of Contents **Page no.**

Table of contents (cont.) **Page no.**

Table of contents (cont.) **Page no.**

Table of Contents **Page no.**

List of tables **Page no.**

Chapter 2

Chapter 3

List of tables (cont.) **Page no.**

List of tables (cont.) **Page no.**

List of figures **Page no.**

List of figures (cont.) **Page no.**

Chapter 1. Introduction

Respiration

In plants, all living cells require access to oxygen to perform aerobic respiration. The product of aerobic respiration, adenosine triphosphate (ATP), is produced by releasing the energy stored in the chemical bonds of sugars (mainly glucose) produced via photosynthesis. The energy stored in ATP is then used for cellular growth and maintenance, as well as nutrient uptake and metabolism. Under normal, non-oxygen-limiting conditions, mitochondrial oxidative phosphorylation is the favored metabolic pathway and has the potential to produce 36 ATP molecules from each molecule of glucose (Fox et al., 1994). The less efficient glycolysis and fermentation pathways are promoted when oxygen becomes limiting (hypoxia, anoxia). Fermentation produces only two ATP molecules for every glucose molecule but allows plants to continue the production of ATP in the absence of oxygen (Catalanotti et al., 2013). Fermentation also results in the buildup of waste products, primarily ethanol, which can be toxic to plant cells (Fry & Huang, 2004). If fermentation continues for an extended period, the energy cost and the resulting buildup of harmful products can ultimately lead to cell death. Therefore, access to oxygen within the soil or growing medium is essential for vigorous plant growth and providing ample oxygen to a plant root system is a constant concern in agricultural and horticultural production.

Soil oxygen

In addition to a shift from aerobic to anaerobic respiration, low soil-oxygen levels also change plant morphology. Low soil-oxygen levels promote the growth of longitudinal aerenchyma channels that facilitate oxygen transport from plant shoots to roots (Yamauchi et al., 2013). Aerenchyma formation occurs in many plants, including creeping bentgrass (*Agrostis* *stolonifera* L.), tall fescue (*Schedonorus arundinaceous*), bermudagrass (*Cynodon dactylon*), rice *(Oryza sativa*), maize *(Zea mays*), barley (*Hordeum vulgare*), and wheat (*Triticum aestivum*) (Li et al., 2001; Jiang & Wang, 2006; Yamauchi et al., 2013; Xiao & Jespersen, 2019). The development of adventitious roots, plant roots that develop from any non-root tissue, has also been reported to increase in many plant species in low-oxygen environments to access as much oxygen as possible (Ayi et al., 2016). For aerial plant parts, access to oxygen is rarely a limiting factor for healthy growth and development due to the large number of stomata and intercellular spaces in the leaves of plants (Vartapetian, 1978) and the high concentration of oxygen (21%) in the atmosphere. Access to oxygen for plant root systems is primarily limited to oxygen present within the soil. Molecular oxygen can be contained in the soil atmosphere or dissolved in soil solution (Pepper & Brusseau, 2019). Unlike atmospheric air, the underlying soil air will often have a higher concentration of carbon dioxide and a lower concentration of oxygen (Howe & Smith, 2021).

The lower oxygen concentration in the soil atmosphere compared to atmospheric air can be further compounded as plant roots are in constant competition with macro and microorganisms, as well as redox reactions, for access to available soil oxygen (Howe & Smith, 2021). According to Ponnamperuma (1984), as soils are cut off from the diffusion of oxygen, the redox potential of the soil will decrease. Reduced soil redox potential will result in changes in concentrations of certain essential plant nutrients, such as denitrification, the conversion of nitrate to nitrite, and ultimately N_2 gas. Low soil redox potential will also result in the microbial reduction of iron, sulfate, and manganese. In well-aerated soils, nitrate, sulfur, manganic and ferric compounds are generally stable (Ponnamperuma, 1984).

As oxygen in the root zone is consumed, replenishment depends on atmospheric oxygen diffusion into the soil, commonly referred to as the oxygen diffusion rate. Soil oxygen-diffusion rates are affected by the physical properties of the soil, most notably, the size and distribution of soil pores (Hillel, 1980). A soil's percentage of sand, silt, and clay influences the size and distribution of soil pores. Sandy soils typically contain mostly large soil pores (macropores), which allow for high oxygen diffusion rates and water infiltration into the soil. As the percentage of clay in soil increases or as soils become compacted from human activity, soil pore size is reduced (Hillel, 1980). This reduction in soil pore-size results in a greater percentage of fine soil pores (micropores), which do not permit air and water flow as freely as macropores (Hillel, 1980).

Sandy soils are preferred for use as golf course putting green root zones due to their resistance to compaction and rapid water drainage potential (Lunt, 1956). The United States Golf Association's recommendations for a method of putting green construction require greater than 60% of the putting green root zone to be composed of medium to coarse sand with particle sizes ranging from 0.25 to 1.0 mm in diameter (U.S. Golf Association, 2018). Even with the use of sand-based putting green root zones, reducing compaction and increasing the aeration of putting green soils is a common concern for golf course superintendents. Golf course superintendents often implement various methods for increasing soil oxygen or reducing compaction in putting green root zones, such as core or solid-tine aerification and sand topdressing. Core aerification reduces compaction, removes soil organic matter, and creates channels to facilitate gas exchange and water infiltration (Christians et al., 2017). However, core aerification is time intensive, disrupts the putting surface, is unpopular with golfers, and can reduce income to a golf facility. Solid-tine aerification and sand topdressing are less disruptive means of increasing soil oxygen but can still be time-intensive and bothersome to golfers. Other less commonly employed methods of increasing soil oxygen include direct injection of pressurized air into the soil (Air2G2, Foley Company, Prescott, WI), direct injection of water into the soil profile (HydroJect, The Toro Company, Bloomington, MN), and the installation of subsurface aeration systems powered by a vacuum blower (SubAir, SubAir Systems LLC, Graniteville, SC). Regardless of the soil aeration method chosen, the costs and inconveniences associated with the previously mentioned methods make alternative, less disruptive aeration methods worthy of exploration. One such means of delivering oxygen to a root zone could be through the water already being used for putting green irrigation by greatly increasing the dissolved oxygen (DO) content of the irrigation water.

Dissolved oxygen

Dissolved oxygen refers to molecular oxygen (oxygen gas) dissolved in any liquid that does not make up the molecular structure of that liquid. The bonded oxygen that makes up the molecular structure of water does not contribute to DO content. According to the National Field Manual for Collection of Water Quality Data, the concentration of DO in water is affected by many factors, including ambient temperature, atmospheric pressure, and ionic strength of the water body. Sources of DO in water include atmospheric aeration facilitated by wind and the photosynthetic activity of aquatic plants. Sinks of DO in water include respiration, aerobic decomposition processes, ammonia nitrification, and other chemical and biological reactions. The presence of DO in aquatic systems is necessary for the survival and growth of many aquatic organisms and is used as an indicator of the health and geochemical quality of surface water and groundwater systems (U.S. Geological Survey, 2020).

Dissolved oxygen is typically measured in mg L^{-1} and can vary greatly depending on the specific water source. Water can be in a state of undersaturation, saturation, or supersaturation of DO (Webb, 2021). Dissolved oxygen saturation occurs when a volume of water holds as many dissolved oxygen molecules as it can in equilibrium with the atmosphere. At this stage, the percentage of oxygen in the water would be equivalent to the percentage of oxygen in the atmosphere, and the water would be considered 100% saturated with DO (Webb, 2021). As water depth increases, DO can remain below 100% saturation due to respiring aquatic organisms and aerobic microbial decomposition. At lower depths, the water is unaffected by wind or atmospheric aeration, and the lack of sunlight results in an inability to support aquatic plant growth, resulting in the under-saturation of DO (Webb, 2021). Supersaturation (above 100% DO saturation of water) can occur through copious amounts of DO released by aquatic plants during photosynthesis, natural or mechanical aeration, or rapid changes in water temperature. The saturated DO content of a body of water depends upon water temperature, water salinity, and barometric pressure (Boyd, 2019). Water temperature and water salinity have an inverse relationship with DO saturation; as water temperature or water salinity increases, the ability of the water to dissolve oxygen is reduced. Barometric pressure directly correlates with DO saturation; as the pressure increases, DO solubility increases (Boyd, 2019). For reference, fresh water at sea level (1 atm) with a temperature of 20 °C contains approximately 9.1 mg L^{-1} DO at 100% saturation.

Oxygation

Enhancing the amount of DO in irrigation water has been attempted in the past to improve crop yield and water use efficiency, with variable results. Nonetheless, scientists have shown increased yields of 10-20% when crops were irrigated with oxygenated water (Du et al., 2018). Oxygation refers to the irrigation of crops with aerated water (containing increased dissolved oxygen levels). Water for oxygation is typically aerated through air injection using the Venturi principle or hydrogen peroxide delivered to the soil (Bhattarai et al., 2005). The aerated water is commonly applied through subsurface drip irrigation (SDI) (Bhattarai et al., 2005). Pendergast et al. (2013) investigated the effects of oxygenated SDI on the growth of cotton (*Gossypium hirsutum* L.) in a vertosol over seven years. They showed a 10% increase in cotton yield and a 7% increase in water-use efficiency. Bhattarai et al. (2004) increased the biomass of zucchini (*Cucurbita pepo*), soybean (*Glycine max*), and cotton in heavy clay soils using oxygenated SDI, attributing the increase to aeration-induced enhanced root function. Air-injected SDI has also increased bell pepper growth (*Capsicum annuum*) in sandy loam soil. Bell pepper count, weight, and root mass were all increased by aerated SDI compared to the unaerated control (Goorahoo et al., 2002). Oxygation through SDI increased pineapple (*Ananas comosas*) fresh biomass and dry matter weight in a loamy sand soil (Chen et al., 2011). Previous research on oxygation has primarily been conducted with agricultural crops and focused on using SDI aeration by forced air injection. Newer technologies utilizing nanobubble oxygenation could increase dissolved oxygen levels in treated water compared to standard water aeration methods.

Nanobubbles

Nanobubbles (NBs) are gas-filled cavities in a liquid that are found dispersed within solution (bulk NBs) or at the interface between a liquid medium and a solid substrate (surface NBs) (Wang et al., 2017). Since the current research investigated the effects of bulk NBs on the DO content of treated water, the discussion of NBs will be limited to bulk NBs. Some disparity exists concerning the nomenclature and size of NBs. Temesgen et al. (2017) and Alheshibri et al. (2016) propose using the term NB to describe bubbles with a diameter of less than one μm.

Throughout scientific literature, the term NB and ultrafine bubble appear to refer to bubbles in the same size range and exhibiting similar properties. Early evidence of the presence of bubbles less than one µm in diameter was reported in seawater by Johnson and Cooke (1981). Since then, most studies involving NBs have focused on NB generation, detection, and characterization (Temesgen et al., 2017).

Nanobubbles have a large surface area per unit volume due to their small size, with a corresponding concentration as high as one hundred million to ten trillion bubbles per milliliter of liquid (Atkinson et al., 2019). Nanobubbles allow for high gas dissolution rates in liquids due to greater internal pressure in the bubble than their environment and high stagnation in the liquid phase (Ushikubo et al., 2010). Additional studies suggest that NBs exhibit a long residence time in solution owing to a negatively charged surface (zeta potential). This surface charge prohibits the coalescence of bubbles, which is characteristic of larger bubbles, which coalesce and rise to the surface (Ushikubo et al., 2010, Takahashi et al., 2007). Nanobubbles have been reported to remain in an aqueous solution for weeks and even months (Azevedo et al., 2016; Duval et al., 2012).

Nanobubbles are created using various methods, including cavitation, electrolysis, and membranes. According to Phan et al. (2020), the cavitation method of NB formation results in the formation of cavities when the homogenous liquid phase undergoes a phase change due to a sudden reduction of pressure below a particular critical value. The sudden reduction in pressure can be achieved through different methods relating to a fluid flow or an acoustic field, referred to as hydrodynamic and acoustic cavitation, respectively. Zhu et al. (2016) state that the creation of NBs through the electrolysis of water involves the decomposition of water into hydrogen and oxygen gases caused by an electric potential. If the concentration of the produced gas reaches the supersaturation level in the anodic and cathodic streams of the bulk water, NBs can be generated. Membranes create NBs by acting as the medium for liquid and gas dispersion, where the gas phase is pressed through the pores of the applied membrane into a flowing aqueous phase (Phan et al., 2020). Nanobubble generation is a complex physiochemical process that depends on many factors, including temperature, applied pressure, electrolyte concentration, concentration and type of dissolved gas in solution, and concentration and type of surfactant (Phan et al., 2020). Several gases are commonly used to create NBs, including oxygen, air, ozone, carbon dioxide, and nitrogen, each utilized for their specific application (Phan et al., 2020).

Nanobubbles have a wide range of potential applications, including but not limited to wastewater treatment, mining, medical, and agricultural and horticultural industries. Nanobubbles are being studied for their applications in the removal of pollutants from water by various methods, including non-conventional flotation, wastewater treatment with ozone, surface cleaning, biological treatment, and aeration of wastewater (Azevedo et al., 2019). Nanobubbles have been demonstrated to be effective at treating mining effluent for the removal of heavy metals and have been shown to facilitate froth flotation of fine coal particles (Fan et al., 2013; Vicente & Flores, 2017). In the medical field, Bhandari et al. (2017) demonstrated the ability of oxygen NBs to reverse hypoxia in tumors and suggested that oxygen NBs could serve as an adjuvant to standard cancer treatments.

Horticultural applications for NBs have also been investigated. Ebina et al. (2013) compared the effects of air-NB treated water to untreated water on the growth of hydroponically cultured *Brassica campestris*. After four weeks, plants grown in air-NB-treated water had greater height, leaf length, and aerial fresh weight than untreated water. Wu et al. (2019) investigated NB-oxygenation to improve the mineralization of organic fertilizer and enhance crop growth in organic tomato production. The authors compared NB oxygenation to traditional pump-aerated oxygenation and a control group through soil incubation and soil column experiments. They reported a tomato yield increase from the NB-oxygenated treatment group of 23% and an increase from the traditional pump-aerated method of 17%, compared with the control. Multiple studies have also reported that NBs promote faster seed growth and germination times (Liu et al., 2016a; Liu et al., 2016b; Liu et al., 2017). Wang et al. (2021) reported enhanced nutrient absorption, a 25% reduction in fertilizer use, and increased yield when irrigating rice with air-NB water. The effects of NB-oxygenated irrigation water have not yet been investigated on plants grown in more porous root zones such as sand-based golf course putting greens.

Rationale

Creeping bentgrass (*Agrostis stolonifera* L.) is a popular and widely used turfgrass for golf course putting green surfaces due to its fine texture and tolerance of low mowing heights. Creeping bentgrass is a perennial, cool-season turfgrass best adapted for use in cool, humid environments. Primarily due to climate, maintenance of creeping bentgrass putting greens is difficult in the southern United States and much of the transition zone, a geographic region that spans from Oklahoma to the Mid-Atlantic states. This zone is referred to as the transition zone because it is a region where both cool- and warm-season grasses are grown, but it is not well suited for either type of grass. In the transition-zone region of Arkansas, cultural practices and environmental factors can place bentgrass under extreme stress during the summer period, resulting in shallow, poorly rooted stands of turf (Carrow, 1996; Fry & Huang, 2004). Turf stress may be compounded by low root zone oxygen as respiration rates of plants and soil micro- and macro-organisms increase due to increased temperatures and humidity in the summer months (Raich & Schlesinger, 1992).

Another component of creeping bentgrass putting green management, which may affect soil oxygen levels, is organic matter accumulation in the upper root zone. Typically, golf course superintendents manage the accumulation of organic matter using a combination of methods, such as physical removal by hollow-tine core cultivation, vertical mowing, dilution of organic matter by sand topdressing, and reducing fertilizer use to slow plant growth (Glasgow et al., 2005). If the organic matter content of a root zone is allowed to increase continually, macropores can become blocked, affecting root zone physical properties such as hydraulic conductivity, oxygen diffusion, and water retention (Adams & Saxon, 1979). Elevated soil oxygen levels may help increase microbial activity responsible for the decomposition of organic matter, helping to fight excessive thatch accumulation in the upper root zone.

The properties of NBs may allow for increased DO retention times in water, and increased DO retention in water at elevated temperatures. Additionally, the surface charge of NBs and the potential effect of NBs on the surface tension of water may increase irrigation water infiltration rates. Ushida et al. (2012) reported a 10% reduction in surface tension of liquid water treated with air NBs, as measured by the du Noüy ring method. The surface tension of liquid water was even further reduced when NB-treated water was combined with an anionic surfactant. Increased infiltration of irrigation water can be important for irrigating sand-based golf course putting greens, which are prone to developing hydrophobicity. Although NB-oxygenation of irrigation water shows potential for increasing oxygen delivery to plant root systems, little is known about the effects of NB-oxygenated irrigation water on the soil oxygen content and plant growth characteristics of a sand-based creeping bentgrass putting green. Currently, anecdotal evidence claiming increased putting green quality from golf course superintendents remains the only proof that sand-based creeping bentgrass putting greens benefit from the use of NB-oxygenated water.

Objectives

The objectives of this research were to:

- Examine the effect of time and NB-oxygenation on dissolved oxygen retention of potable water.
- Examine the effects of NB-oxygenated water on the infiltration of irrigation water on a sand-based creeping bentgrass putting green.
- Examine the effects of overhead irrigation with NB-oxygenated water on soil oxygen, soil organic matter, and plant health indicators of a sand-based creeping bentgrass putting green.
- Examine the effects of subsurface irrigation with NB-oxygenated water sources on plant performance of creeping bentgrass grown in sand-based lysimeters.

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Chapter 2. Effect of nanobubble-oxygenation on dissolved oxygen content and infiltration rate of irrigation water sources

Abstract

The theoretical properties of nanobubbles (NBs), such as a negative surface charge and large interfacial surface area, allow for highly efficient gas transfer and stagnation time in water and may reduce the surface tension of NB-treated water sources. These properties make NBs unique candidates for addressing issues like root zone oxygen deficiency, common in conventional and hydroponic crop production, and soil hydrophobicity, common on sand-based golf-course putting greens. The theoretical properties of NBs and NB-oxygenation of irrigation water may provide a means of supplying adequate dissolved oxygen (DO) at high nutrient-solution temperatures for hydroponic crop irrigation. Additionally, the reduced surface tension of irrigation water may allow for increased water infiltration in sand-based putting greens. Therefore, the objectives of this research were to confirm the presence of nanobubbles in treated water, determine how time and temperature affect DO retention of NB-oxygenated water, and investigate differences in infiltration rates of different NB-oxygenated irrigation water sources in sand-based putting greens. Nanobubble-oxygenation increased initial levels of DO in water compared to a standard air stone. Nanobubble oxygenation failed to increase DO retention time compared to a standard air stone, regardless of water temperature. The infiltration rate of irrigation water was not affected by NB-oxygenation. Nanobubble-oxygenation remains a method of efficiently oxygenating large volumes of water, although the NBs investigated in the study did not increase DO retention or infiltration rate of irrigation water.

Introduction

Dissolved oxygen

Molecular oxygen can be found in the soil atmosphere or dissolved in soil solution (Scott & Evans, 1955). Providing ample oxygen to a plant root system is a constant concern in agricultural and horticultural production. In agricultural crops, plant root systems may be exposed to oxygen-deficient (hypoxia) conditions within the root zone if soils have poor porosity or become waterlogged (Ponnamperuma, 1984). There are many ways to modify root zones to enhance porosity, including soil amendments, cultivation, the inclusion of cover crops, and no-till methods. However, these can often be expensive and difficult to implement. In addition to these methods, the dissolved oxygen (DO) contained in rainwater or irrigation can be delivered to the soil and used for soil respiratory processes (Chen et al., 2011).

After irrigation events, the root zone can remain saturated, decreasing soil oxygen levels and restricting gas exchange (Niu et al., 2012). This oxygen restriction could be further compounded when irrigating with water containing low levels of DO. Agricultural irrigation ponds often experience reduced DO concentrations during the summer months due to increased water temperature, and the summer also corresponds to the season of heaviest reliance on irrigation water (Hasanoğlu & Göncü, 2018). As warm air and sunlight increase the temperature and productivity of the upper portion of a water body, thermal stratification of irrigation ponds leads to oxygen depletion in the hypolimnion, the lowest layer of a stratified lake. Hypolimnetic oxygenation can be important to long-term irrigation pond health (Beutel & Horn, 1999).

Different from agricultural production, roots of crops produced hydroponically require access to oxygen dissolved in a nutrient solution. In deep-flow-technique production systems, inadequate root aeration can be a problem, as oxygen diffusion can be drastically reduced in stagnant nutrient solutions (Morimoto et al., 1989). Oxygen deficiencies can also be a concern in other methods of hydroponic crop production, such as nutrient-film-technique systems (Giselrod & Kempton, 1983; Jackson, 1980). This problem may be compounded during the summer, as nutrient-solution oxygen solubility is reduced in greenhouses experiencing high-temperature extremes. As nutrient solution temperature increases, there is a direct relation to plant oxygen consumption and an inverse relation to the amount of oxygen dissolved in the solution (Al-Rawahy et al., 2019). Even in cooler climates such as Ontario, Canada, root zone DO levels in a commercial cucumber greenhouse were reported to be as low as 2 mg L^{-1} during the summer months (Zheng et al., 2007).

Enhancing the amount of dissolved oxygen in irrigation water has been attempted in the past to improve crop yield and water use efficiency, with variable results (Du et al., 2018). Nonetheless, scientists have shown increased yields of 10-20% when crops were irrigated with oxygenated water (Pendergast et al., 2013). Identifying a means of oxygenating water for crop irrigation that allows for the retention of DO, especially at elevated temperatures, could be of significant benefit to the horticultural industry.

Infiltration

Soil hydrophobicity is a global problem but most often occurs in sandy soils (Olorunfemi et al., 2014). Sands are the preferred growing medium for putting green root zones due to their resistance to compaction and rapid water drainage potential (Lunt, 1956). The sandy root zone mixture of a putting green constructed to USGA specifications (U.S. Golf Association, 2018), the most popular construction method, can rapidly develop hydrophobicity, especially near the surface of the root zone. This hydrophobicity is caused by individual sand particles that have become coated with organic compounds exhibiting hydrophobic properties (Miller & Wilkinson, 1977; Henry & Paul, 1978). Localized dry spot (LDS) is a term used in the golf course industry to define irregularly shaped areas of desiccated, brown turf. The decline in turf quality associated with LDS results from soil that has become hydrophobic and resists wetting from irrigation and rainfall (Beard & Beard, 2005). Localized dry spot can be a major issue during summer months when uniform moisture distribution in the root zone is necessary for the playability and aesthetics of an intensely managed putting surface.

In turfgrass culture, research surrounding soil water repellency and the use of wetting agents to increase water infiltration in hydrophobic soils has been expanding, beginning in the late 1990s and early 2000s (Fidanza et al., 2020). Wetting agents have become a popular and reliable tool to mitigate damage caused by localized dry spot. Wetting agents are part of a chemical group called surfactants or "Surface Active Agents". Surfactants effectively reduce the surface tension of water by lowering its cohesive properties, allowing water to penetrate the surface of a hydrophobic soil (Cisar et al., 2000, Kostka, 2000). Because wetting agents do not correct hydrophobicity in the soil but rather temporarily mask the symptoms of LDS, they are used frequently throughout the summer months (Jacobs and Barden, 2018). Any means of reducing the surface tension of water for irrigation of sand-based putting greens could be beneficial as part of a well-rounded LDS prevention or management program. The properties of nanobubble-oxygenated irrigation may allow for increased water infiltration rates.

Nanobubbles

Nanobubbles (NBs) are gas-filled cavities in a liquid that are found dispersed within solution (bulk NBs) or at the interface between a liquid medium and a solid substrate (surface NBs) (Wang et al., 2017). Although some disparity exists concerning the nomenclature and size of NBs, Temesgen et al. (2017) and Alheshibri et al. (2016) propose using the term nanobubble to describe bubbles with a diameter of less than one μm. Early evidence of the presence of bubbles less than one μm in diameter was reported in seawater by Johnson and Cooke (1981). Since then, most studies involving NBs have focused on NB generation, detection, and characterization (Temesgen et al., 2017).

Nanobubbles exhibit several unique properties. Because of their small size, NBs have a large surface area per unit volume, with a corresponding concentration as high as one hundred million to ten trillion bubbles per milliliter of liquid (Atkinson et al., 2019). Nanobubbles allow for high gas dissolution rates in liquids due to higher internal pressure in the bubble than their environment and high stagnation in the liquid phase (Ushikubo et al., 2010). Additional studies suggest that nanobubbles exhibit a long residence time in solution owing to a negatively charged surface (zeta potential). This surface charge prohibits the coalescence of bubbles, which is characteristic of larger bubbles, which coalesce and rise to the surface (Ushikubo et al., 2010, Takahashi et al., 2007). Nanobubbles have been reported to remain in an aqueous solution for weeks and even months (Azevedo et al., 2016; Duval et al., 2012). Nanobubbles have also been experimentally observed to reduce the surface tension of water. Ushida et al. (2012) reported a 14% reduction in the surface tension of water as measured by the du Noüy ring method. The reduction in surface tension was further increased when combined with an anionic surfactant (Ushida et al., 2012).

Nanobubbles are created using various methods, including cavitation, electrolysis, and membranes. According to Phan et al. (2020), the cavitation method of NB formation results in the formation of cavities when the homogenous liquid phase undergoes a phase change due to a sudden reduction of pressure below a particular critical value. The sudden reduction in pressure can be achieved through different methods relating to fluid flow or an acoustic field, referred to as hydrodynamic and acoustic cavitation, respectively. Zhu et al. (2016) state that the creation of nanobubbles through the electrolysis of water involves the decomposition of water into hydrogen and oxygen gases caused by an electric potential. If the concentration of the produced gas reaches the supersaturation level in the anodic and cathodic streams of the bulk water, NBs can be generated. Membranes create NBs by acting as the medium for liquid and gas dispersion, where the gas phase is pressed through the pores of the applied membrane into a flowing aqueous phase (Phan et al., 2020). Nanobubble generation is a complex physiochemical process that depends on many factors, including temperature, applied pressure, electrolyte concentration, concentration and type of dissolved gas in solution, and concentration and type of surfactants (Phan et al., 2020). Several gases are commonly used to create NBs, including oxygen, air, ozone, carbon dioxide, and nitrogen, each utilized for specific applications (Phan et al., 2020).

The properties of NBs may allow for increased DO retention times in water, and increased DO retention in water at elevated temperatures, which is important for crop production in systems with seasonal and diurnal irrigation water temperature fluctuations. Additionally, the surface charge of NBs and the potential effect of NBs on the surface tension of water may increase infiltration rates of irrigation water, especially in soils prone to developing hydrophobicity. The objective of our research was to confirm the presence of NBs in treated water, characterize how time and temperature affect the DO retention of NB-oxygenated water, and investigate differences in infiltration rates of irrigation water sources, including water oxygenated with NBs. It was hypothesized that NB-oxygenation would result in more nanoscopic particles per ml of water and longer DO retention compared to traditional means of water oxygenation, regardless of water temperature. Additionally, we hypothesized that NB-oxygenation of water would increase infiltration rates compared to untreated water in a sand-based putting green.

Materials and Methods

Dissolved oxygen study

Research was conducted at the University of Arkansas Rosen Alternative Pest Control Center in Fayetteville, AR. All water originated from the same source, supplied by Beaver Water District (Lowell, AR). Four treatments included:

- Proprietary membrane-based nanobubble injection system (Nano Bubble Technologies, Sydney, NSW, AU) delivering industrial grade oxygen (Airgas, Radnor, PA) injected at a rate of 1.5 L min^{-1} (**NBT**)
- Proprietary membrane-based nanobubble injection system (Moleaer Inc., Torrance, CA) delivering industrial grade oxygen (Airgas, Radnor, PA) injected at a rate of 1.5 L min^{-1} (**Moleaer**)
- 15-cm aquarium air stone (TopFin, United Pet Group, Atlanta, GA) delivering industrial grade oxygen (Airgas, Radnor, PA) injected at a rate of 1.5 L min^{-1} (**Air Stone**)
- Untreated control - Standard potable water (**Control**)

Experimental units were collected in 3.8-L glass jars with circular mouths that measured 5 cm in diameter, allowing for 81 cm^2 of water exposed to the atmosphere. Nanobubble-oxygenated treatments were cycled through respective NB injection systems, each delivering 1.5 L min^{-1} of industrial-grade oxygen to a water volume of 379 L, cycling at a rate of 42 L min^{-1} for a period of 1 hr. The 15-cm aquarium stone was placed directly into each 3.8-L container and was allowed to oxygenate for one hr. Containers were sealed with an airtight cap and transported to a growth chamber. Temperature-controlled growth chambers (Conviron E7, Winnipeg, MB) were set to maintain temperatures of 20, 30, and 40 °C. The DO content of each treatment was measured at 0 and 24 hours, and then every 24 hours until DO levels returned to that of the untreated control. Dissolved oxygen content was measured using a portable DO meter (Hannah Instruments, Model HI98193, Woonsocket, RI) and a Clark-type polarographic DO probe with a polytetrafluoroethylene polymer membrane cap (Hannah Instruments, Model HI764073, Woonsocket, RI).

The experimental design was a randomized complete block with four replications of each water treatment, with two separate experimental runs of each growth chamber temperature. Blocking was utilized to account for any minor variations that may have existed within the growth chamber. Testing of temperature effects was not conducted due to output limitations of our specific nanobubble injection systems, which resulted in extended periods between generated batches of water that would have allowed for inconsistencies between batches placed in growth chambers of different temperatures. Therefore, all water treatment comparisons are only made within a given temperature. Dissolved oxygen data were subjected to a repeated measures analysis of variance (ANOVA) in SAS (SAS version 9.4; SAS Institute, Cary, NC) using PROC MIXED. Slicing was performed to detect specific times at which treatment means were significantly different. Where appropriate, treatment means were separated using Fisher's protected least significant difference (LSD) at the $\alpha = 0.05$ level.

NanoSight analysis

To quantify and characterize the nanobubbles present in treated water, nine replications of each treatment, excluding the Air Stone, were analyzed at the Vanderbilt University Institute of Nanoscale Science and Engineering (Nashville, TN) on three separate occasions using a

NanoSight NS300 (Malvern Panalytical, Salisbury, United Kingdom) equipped with a scientific complementary metal-oxide semiconductor camera and a red laser. The Moleaer unit was no longer available on the third sampling, so the analysis only compared the NBT injection system to the Control. NanoSight analysis determined the total concentration of nanoscopic particles per milliliter of water, the mean size and standard deviation of size of nanoscopic particles, and the D10, D50, and D90 of each sample, which signify the particle diameter below which 10, 50, and 90% of the total volume in the sample was contained. Data were subjected to ANOVA in SAS (SAS version 9.4; SAS Institute, Cary, NC) using PROC MIXED. Where appropriate, treatment means were separated using Fisher's protected LSD at the $\alpha = 0.05$ level.

Infiltration study

A membrane-based NB-injection system (Nano Bubble Technologies, Sydney, AU) was used to oxygenate both potable water from the Beaver Water District (Lowell, AR) and irrigation pond water from the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR (36.09º N, 94.17º W). The two main effects of water source (Potable or Pond) and NB-oxygenation (NB-oxygenated or Untreated) were arranged in a 2 × 2 factorial totaling four treatments that included the following:

Potable water treatments:

- Oxygenated using the NBT system (**NBT - POT**)
- Untreated (**POT**)

Irrigation-pond water treatments:

- Oxygenated using the NBT system (**NBT - PND**)
- Untreated (**PND**)

Nanobubble-oxygenated treatments were cycled through the NB injection system delivering 1.5 L min^{-1} of industrial-grade oxygen to a water volume of 94 L, cycling for one hour at a rate of 26 L min^{-1}.

On 6 Jan. 2021 and 5 Jan. 2022, infiltration measurements were conducted on a sand-based creeping bentgrass (*Agrostis stolonifera* L.) putting green. Measurements were conducted utilizing sixteen 1.2- by 1.2-m plots. Plots were being used concurrently for a separate but related trial, focused on irrigation with each of the four water treatments used in this study (see Chapter 3). Each plot represented one of four replications of each of the four water treatments arranged in a randomized complete block design. From May through September, each plot was irrigated exclusively with the water treatment assigned to that plot. Monthly wetting agent applications of Immerse GT (Amega Sciences Inc., Lakeland, FL) were made at a rate of 19 L ha^{-1} to the entire experimental area during the spring and summer seasons but were discontinued on 30 September in each season. Two random locations within each plot were selected for infiltration measurements.

Measurements were conducted using the procedure described by Jacobs et al. (2015). A double-ring infiltrometer (model IN7-W, Turf-Tec International, Tallahassee, FL), with a 15-cm inner-ring diameter, was installed manually to a depth of approximately 2.5 cm, so that no water leakage occurred from the perimeter of the outer ring. Once the infiltrometer was installed, the soil volumetric water content in the 0- to 6-cm depth interval was measured in triplicate by time-domain reflectometry using a TDR 300 soil moisture meter (Spectrum Technologies, Aurora, IL) in the outer ring of the infiltrometer. The outer ring of the infiltrometer was first filled with water, followed by filling the inner ring. All water used corresponded to the water treatment associated with that plot. The height of the water column inside the inner ring was recorded immediately after the inner ring was filled to represent time zero. The height of the water column in the inner ring was subsequently recorded at 1, 2, 3, 4, 5, 8, 10, 12, 14, 16, 18, and 20 minutes thereafter. If all water in the inner ring infiltrated before the 20-minute measurement period ended, then the time into the infiltration measurement when all the water infiltrated was recorded. The overall infiltration rate (cm hr^{-1}) over the 20-minute measurement period, or over the time it took for complete infiltration to occur if the time was less than 20 minutes, was calculated for each infiltration measurement. Volumetric water content was subjected to ANOVA, and infiltration rate was subjected to two-factor (Water and Nano) ANOVA in SAS (SAS version 9.4; SAS Institute, Cary, NC) using PROC GLIMMIX. Where appropriate, treatment means were separated using Fisher's protected LSD at the $\alpha = 0.05$ level.

Results and Discussion

NanoSight analysis

NanoSight analysis, which uses a camera to analyze laser light scattered by nanoparticles, confirmed the existence of NBs in NB-oxygenated water. However, the number of nanoscopic particles detected was inconsistent between treatments and analyses (Tables 2.1, 2.2, 2.3). Each NB treatment significantly increased nanoparticle concentration compared to Control water in only one of three analyses, even though nanoparticle concentrations were numerically greater for NB treatments in every analysis (Tables 2.1, 2.2, 2.3). Mean nanoparticle size, standard deviation, and size distribution were inconsistently affected by NB-oxygenation (Tables 2.1, 2.2, 2.3). Detected particle size was generally greater and more variable in NB-oxygenated water compared to the Control but never differed between NB treatments. The size of NBs created through porous membranes is affected most notably by the flow rate of the water in which they are created (Ulatowski & Sobieszuk, 2018). Increased particle size variability in NB treatments compared to the Control was surprising due to the generation of NBs occurring through a porous membrane of fixed pore size with water at a constant flow rate. Because proprietary technology was used to conduct this research, pore sizes of the specific membranes used were unavailable. It is possible, however, that a range of pore sizes in the membranes resulted in increased variability of nanoscopic particles compared to that detected in the Control.

It is important to note the limitations of our analyses confirming the presence of NBs in treated water. Because this research was designed to have direct applicability for practitioners who likely do not use ultrapure water for irrigation, potable water sources rather than ultrapure water sources were used for NanoSight analysis. Because ultrapure water sources were not used in our experiments, we cannot say with certainty that all particles detected were NBs, due to the difficulty of distinguishing between nanoparticles and NBs (Eklund, 2019; Yasui et al., 2019). The limitation of our work is also a limitation for potential end users of NB technology. Detection limitations are a significant challenge for NB detection in nonpure water sources. Because irrigation water and hydroponic nutrient solutions are not ultrapure water sources, the inability to distinguish between nanoparticles and NBs is an important limitation for golf course superintendents or hydroponic producers wanting to confirm the functionality of their systems by the detection of NBs in treated water. This limitation should be strongly considered before purchasing and installing NB injection systems based on the current state of measurement technology.

Indirect measurements like the induced coalescence method, the light scattering method, and DO reverse estimation are the most accessible methods for monitoring the existence of NBs in treated water (Kim et al., 2018). The induced coalescence method requires applying irradiating ultrasonic waves to the water, which makes this method an additional challenge for users of NB technology (Kim et al., 2018). The light scattering method is the simplest method for determining the existence of sub-micron bubbles, but the scattering of light could be affected by turbid water sources used in golf course irrigation and hydroponic production (Kim et al., 2018). Induced coalescence and light scattering do not indicate the size or number of bubbles created, simply that bubbles of less than 1 µm are present in the water. Dissolved oxygen reverse estimation allows for bubble size estimation but requires monitoring the DO content of treated water under three different pressures, which can be a time-intensive process, not well suited for real-time water analysis (Kim et al., 2018). Due to the difficulty associated with DO reverse estimation, this method should not be considered for real-time analysis of NB-treated water. Kim et al. (2018) state that DO concentration analysis confirmed the presence of sub-micron bubbles, but it was unclear how the authors measured DO and how it confirmed the existence of sub-micron bubbles. Until efficient, reliable detection of NBs becomes accessible for impure water sources, confirming the existence of NBs in treated water will continue to hinder the widespread use of NB technology.

Dissolved oxygen study

Nanobubble-oxygenated treatments produced greater initial DO concentrations compared to the Air Stone immediately after generating the oxygenated water (Figs. 2.1, 2.2, 2.3). In both 20 °C experimental runs, NBT contained the greatest initial DO concentration compared to all other treatments (Fig. 2.1). The Air Stone treatment resulted in a greater initial DO concentration compared to Moleaer in one experimental run of the 30 °C growth chamber but was never greater than NBT (Fig. 2.2). In both 40 °C experimental runs, NB-treatments contained greater initial DO concentrations compared to the Air Stone (Fig. 2.3). Initial dissolved oxygen concentrations in NB treatments were similar (typically >30 mg L^{-1}) but slightly lower than what was observed by Langenfeld and Bugbee (2021), who circulated 100 L of water through a membrane-based NB-injection system.

Because the focus of this work was to investigate the retention of DO rather than the effect of water temperature at generation on oxygen dissolution levels, it should be noted that the water temperature at the time of generation was approximately 15 ℃, which is less than the growth chamber temperatures in which the water was placed. Because water holds less DO as it warms, it is reasonable to assume that had the water been generated at a temperature of 40 °C, a decrease in initial DO concentration for all water treatments would have been observed compared to water generated at 30 or 20 °C. Further investigation into the effect of water temperature on DO content at the initial generation of NB-oxygenated water will help with understanding the ability of NB-injection systems to oxygenate water at elevated temperatures. Because irrigation water used in the horticultural and agricultural industries is subjected to diurnal and annual water temperature fluctuations, the ability to oxygenate water at high temperatures is important for the applicability of NB technology to the horticultural and agricultural industries.

Nanobubble-oxygenated water treatments did not result in increased retention time of DO compared to the Air Stone treatment at any growth chamber temperature in either experiment. Due to the greater initial DO concentrations observed in NB-oxygenated water treatments, a significant interaction of Oxygenation Method × Time was detected for every growth chamber temperature in each experimental run (Table 2.2). Dissolved oxygen concentrations in all oxygenated treatments dropped to levels similar to the Control after 240, 120, and 96 hrs, corresponding to the 20, 30, and 40 °C growth chambers, respectively (Figs. 2.1, 2.2, 2.3).

Although no statistical comparisons were made between temperatures, as expected, increased growth chamber temperature resulted in faster DO loss.

One explanation for DO loss from NB-oxygenated water occurring faster than what was hypothesized is a weak negative surface charge (zeta potential) on the NBs generated in this study. Zeta potential is a critical parameter when discussing bubble stability (Han et al., 2004; Han et al., 2006; Hewage, 2020). Nanobubbles created in pure water, free of electrolytes, have high absolute zeta potentials that create repulsion forces, reducing bubble coalescence. Because there is an inverse relationship between the electrolyte concentration of water and the magnitude of negative NB surface charge, increasing electrolyte concentration in water decreases the magnitude of the bubble zeta potential (Han et al., 2004; Han et al., 2006). More specifically, cations with high valency have the potential to neutralize the bubble charge (Hewage, 2020). Although the zeta potential of bubbles was not measured directly in our research, the presence of electrolytes in our water source (Appendix A-1) may have reduced the absolute zeta potentials of generated NBs, resulting in reduced DO stability in the NB-oxygenated water.

Nanobubbles have been observed to be stable in solution for multiple weeks (Azevedo et al., 2016; Duvall et al., 2012). The stable NBs observed by Azevedo et al. (2016) were generated using air injection, rather than pure oxygen, in ultrapure water (filtered and deionized). The lack of electrolytes in the treated water likely played a role in the long-term stability of the NBs reported by Azevedo. Stable NBs observed by Duvall et al. (2012) were reported as being generated in ultrapure water by a precise, vigorous shaking process in the presence of purified air. The ultrapure water contained added monovalent ions of known concentrations. Bubble stability was greatest in solutions with the lowest concentration of ions.

The lack of multivalent ions in the treated water investigated by Duval et al. (2012) also likely facilitated long-term bubble stability. The water used in our research originated as tap water from the Beaver Water District (Lowell, AR) (Appendix A-1), and pure oxygen was used to create NBs. The difference in gases and solutes between our experiment and those of Azevedo et al. (2016) and Duvall et al. (2012) likely resulted in differences in bubble stability; therefore, oxygen loss occurred more rapidly than expected.

Because water used for irrigation and hydroponic nutrient solutions contains electrolytes not found in pure or deionized water, NB stability may be compromised in field applications of NB technology. Additionally, DO loss from flowing NB-oxygenated water occurs rapidly, making the continuous generation of NBs necessary to maintain elevated levels of DO in treated water (Langenfeld & Bugbee, 2021). The rapid loss of DO observed in flowing water by Langenfeld and Bugbee (2021) could also impact the use of NB-treated water for use in golf course irrigation systems. The distance the NB-treated water must travel from an NB generator to reach its intended location, coupled with the atomization of the water flowing through an irrigation head and nozzle, could also result in rapid DO loss.

Infiltration

Infiltration of rainfall or irrigation into the soil can be greatly influenced by antecedent soil moisture content (Williams et al., 1998). As soils approach saturation, the infiltration rate decreases due to a lack of pore space and a reduction in the hydraulic gradient. Because ANOVA ($\alpha = 0.05$) determined that antecedent soil volumetric water content did not differ between plots in either year prior to conducting infiltration tests, it was concluded that antecedent soil moisture was not a source of variability for infiltration rates observed in this experiment.

Nanobubble-oxygenation of irrigation water sources failed to increase the infiltration rate of irrigation water sources in our experimental sand-based putting green (Table 2.5). Infiltration rate was only affected in one year by the Water Source treatment (Table 2.5). The infiltration rate for Pond water (12.2 cm hr^{-1}) was significantly faster than Potable water (6.6 cm hr^{-1}). The greater infiltration rate of Pond water compared to Potable water was not replicable and was likely an anomaly caused by the heterogeneous nature of the putting green soil.

Although the surface tension of treated water was not measured directly, indirect measurement of surface tension through the determination of infiltration rates demonstrated that the surface tension of NB-Oxygenated water was not reduced to the degree that would result in faster water infiltration in a sand-based root zone. Jain and Qiao (2017) used non-reactive molecular dynamic simulations of NBs and H_2O-H_2O, O_2-O_2, and H_2O-O_2 interactions to demonstrate that the surface tension of water decreased with an increase in the concentration of dissolved O_2 gas molecules. Therefore, it is logical that the oxygen-NBs present in the water used to conduct our experiments would increase infiltration rates due to a reduction in surface tension. One explanation for why we did not observe increased infiltration rates could be the concentration of solutes in the water used in our research (Appendix A-1).

It is likely that the predicted reduction in surface tension observed by Jain and Qiao (2017) would be altered in water sources that contain more than O_2 and H_2O molecules. According to Yasui et al. (2019), it seems improbable that NBs would reduce the surface tension of water due to the concentration of NBs at the water's surface being four orders of magnitude less than what would be required to produce reductions of surface tension. Additionally, Yasui et al. (2019) attribute the observed reduction in surface tension of NB-treated water by Ushida et al. (2012) to the specific method used (du Noüy Ring Method) for determining the surface tension value. The previous explanation is only offered as a possible reason for the lack of increased infiltration in NB-oxygenated water sources observed in this research. Discussion regarding the effects of using different methods for determining the surface tension of NB-oxygenated water is beyond the scope of this research and will not be discussed further.

It should also be noted that the specific putting green used in our research was not displaying symptoms of LDS, as monthly wetting agent applications had been used to prevent LDS formation before conducting this research. Although wetting agent applications ceased three full months prior to conducting our research, it may still be possible that their application for an entire growing season prior to conducting infiltration measurements masked surface tension reductions attributed to the concentration of NBs in the water. Wetting agents have been shown to persist in the soil over several months, with residual effects of a late-fall wetting agent application observed during green-up the following spring (Bauer et al., 2017; DeBoer et al., 2020). It is possible that the data collected may have differed had this infiltration research been conducted in artificially created hydrophobic sands or on sections of a putting green displaying LDS symptoms. Further research investigating the effects of the specific dissolved gas, concentration and size of NBs in solution, and water source variation on the infiltration of NB-treated water will help elucidate NB-generation methods that maximize surface tension reduction and infiltration rates of treated water.

Conclusions

As we hypothesized, NB-oxygenated water treatments contained a greater nanoscopic particle concentration than control water. Significant NB treatment effects were detected in two of three NanoSight analyses conducted, as each of the two NB treatments significantly increased the concentration of nanoscopic particles in one analysis. It should be noted that a numerical increase in nanoscopic particles compared to control water was detected for both NB treatments in every analysis conducted. Particle size analysis determined that the particle size in NB treatments was generally larger and more variable compared to particle sizes detected in control water. A significant hurdle that currently limits the broad application of this technology is the ability to quickly and accurately measure the nanobubble concentrations in generated water. Until those challenges are overcome, it will be almost impossible for a producer to assess the proper function of NB-injection systems.

This research rejects our hypothesis that NB-oxygenation of irrigation water sources would increase DO retention in treated water. The DO retention-time of water was never affected by NB-oxygenation. Although not statistically compared, as expected, warmer growth chamber temperature resulted in more rapid DO loss from oxygenated treatments, as measured by the number of hours for DO levels to return to the level present in control water. Generally, the NB treatments significantly increased initial concentrations of DO compared to the Air Stone treatment. Despite typically beginning with more DO, the DO retention times of NB treatments did not differ from the Air Stone treatment, as DO in every oxygenated treatment fell to levels present in control water after the same number of hours.

This research rejects our hypothesis that NB-oxygenation of irrigation water sources would increase the infiltration rate of irrigation water in a sand-based root zone. Infiltration rate was never affected by NB-oxygenation. This research demonstrates that NB-oxygenation of irrigation water sources can allow for excellent levels of oxygen dissolution in irrigation water, but other benefits, such as longer DO retention and increased water infiltration rates, may not be observed. Other than efficient gas dissolution, expectations of improved water performance should be tempered and considered prior to investing in a membrane-based NB injection system.

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Table 2.1. NanoSight analysis of the mean, standard deviation, size distribution, and concentration of nanoscopic particles in two water sources oxygenated using nanobubble injection systems and an untreated control.

Effect	Mean (nm)	StDev	D10[b] (nm)	D50 (nm)	D90 (nm)	Concentration (particles ml^{-1})
Moleaer	166.0 a[a]	106.5 a	82.5 a	134.6 a	278.0 a	109,180,011 a
NBT	152.8 a	103.4 a	74.4 a	132.9 a	248.9 a	31,220,003 b
Control	122.0 b	51.6 b	69.8 a	110.9 b	185.5 b	21,540,002 b
LSD P = 0.05	22	35	13.6	11.4	53.5	22,900,241
Treatment Prob (F)	0.0028	0.0081	NS[c]	0.001	0.008	<0.0001

[a]Within columns, values followed by the same letters are not significantly different according to LSD ($\alpha = 0.05$).
[b]D10, D50, D90 correspond to the particle diameter below which 10%, 50% and 90% of sampled nanoparticles were contained.
[c]NS, non-significant at the 0.05 probability level.

Table 2.2. NanoSight analysis of the mean, standard deviation, size distribution, and concentration of nanoscopic particles in two water sources oxygenated using nanobubble injection systems and an untreated control.

Effect	Mean (nm)	StDev	D10[c] (nm)	D50 (nm)	D90 (nm)	Concentration (particles ml^{-1})
Moleaer	151.0 a[a]	57.3 a	85.9 a	149.0 a	217.0 a	32,550,000 a
NBT	140.4 a	74.4 a	77.3 a	123.3 a	224.6 a	30,075,000 a
Control	148.1 a	71.9 a	89.1 a	128.1 a	231.4 a	23,725,000 b
LSD P = 0.05	22	35	13.6	11.4	53.5	19,500,000
Treatment Prob (F)	NS[b]	NS	NS	NS	NS	NS

[a]Within columns, values followed by the same letters are not significantly different according to LSD ($\alpha = 0.05$).
[b]NS, non-significant at the 0.05 probability level.
[c]D10, D50, D90 correspond to the particle diameter below which 10%, 50% and 90% of sampled nanoparticles were contained.

Table 2.3. NanoSight analysis of the mean, standard deviation, size distribution, and concentration of nanoscopic particles in water oxygenated using a nanobubble injection system and an untreated control.

Effect	**Mean** (nm)	**StDev**	**D10**[b] (nm)	**D50** (nm)	**D90** (nm)	**Concentration** (particles ml^{-1})
NBT	205.6 a[a]	89.8 a	115.1 a	188.0 a	313.3 a	161,750,000 a
Control	132.6 b	59.1 b	78.1 b	118.9 b	211.5 b	29,150,000 b
LSD P = 0.05	19.11	17.7	10.8	22.5	27.4	40,400,000
Treatment Prob (F)	<0.0001	0.0053	0.0002	0.0003	0.0001	0.0002

[a]Within columns, values followed by the same letters are not significantly different according to LSD ($\alpha = 0.05$).
[b]D10, D50, D90 correspond to the particle diameter below which 10%, 50% and 90% of sampled nanoparticles were contained.

Table 2.4. Analysis of variance testing the main effects and interaction of time and oxygenation method on dissolved oxygen retention in water. Two experimental runs were conducted in three separate growth chamber temperatures.

Effect	**Growth Chamber Temperature**					
	Experiment 1			Experiment 2		
	20 °C	30 °C	40 °C	20 °C	30 °C	40 °C
	---------- P > F ----------					
Oxygenation method	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Oxygenation method × Time	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 2.5. Analysis of variance testing the main effects and their interaction on the infiltration rate of nanobubble-oxygenated water sources in a sand-based creeping bentgrass putting green.

Effect	Infiltration rate (cm hr^{-1})	
	2021	2022
	--------------- P > F ---------------	
Water source[a]	0.0463	NS[b]
Oxygenation method	NS	NS
Water source × Oxygenation method	NS	NS

[a] Water sources had been used to irrigate experimental plots for a minimum of 5 months prior to conducting experiments.
[b] NS, Nonsignificant at the 0.05 probability level

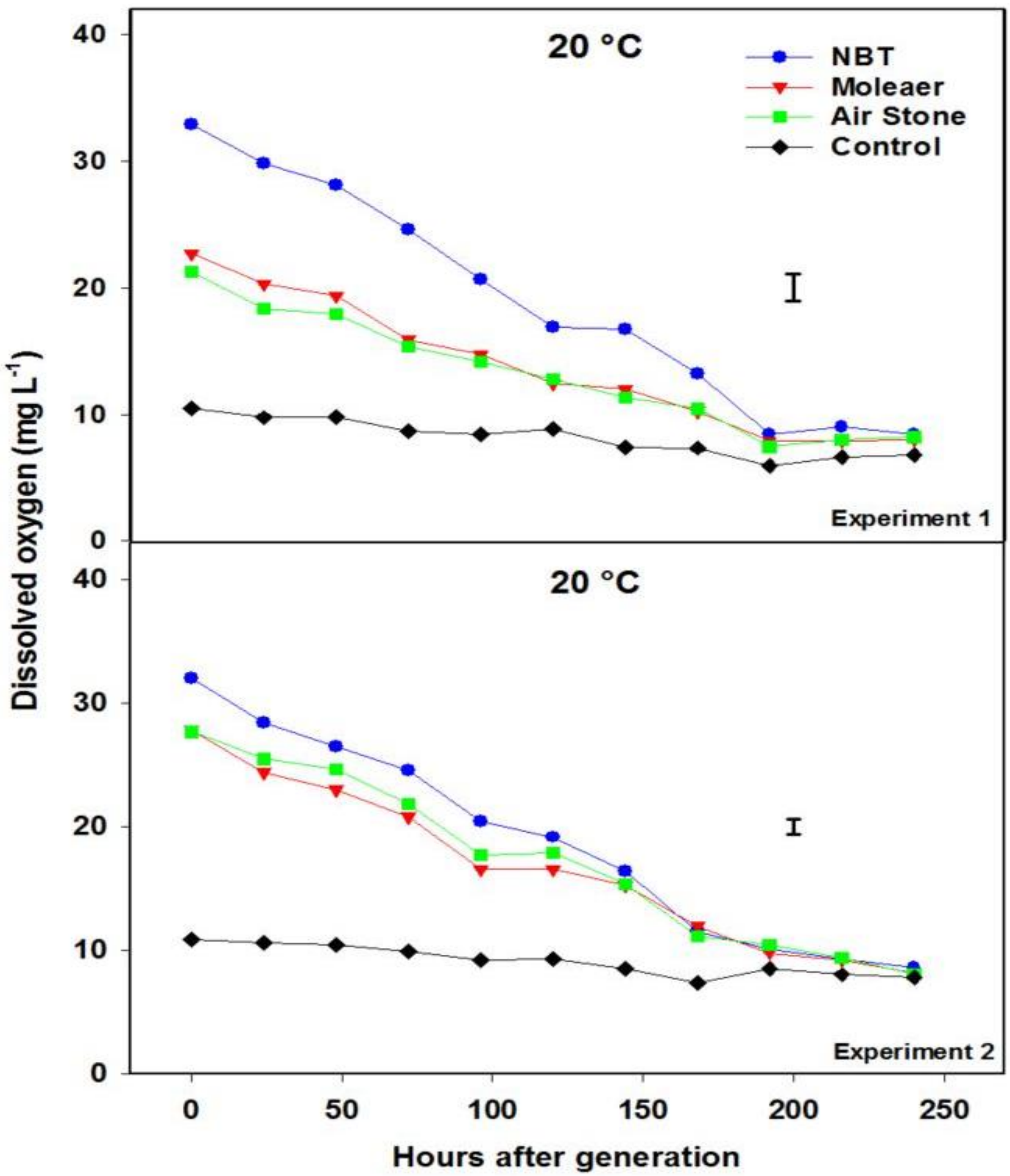

Figure 2.1. Effect of Oxygenation Method × Time on dissolved oxygen retention of three methods of water oxygenation and an untreated control at 20 °C. Error bar represents the least significant difference for comparing means ($\alpha = 0.05$).

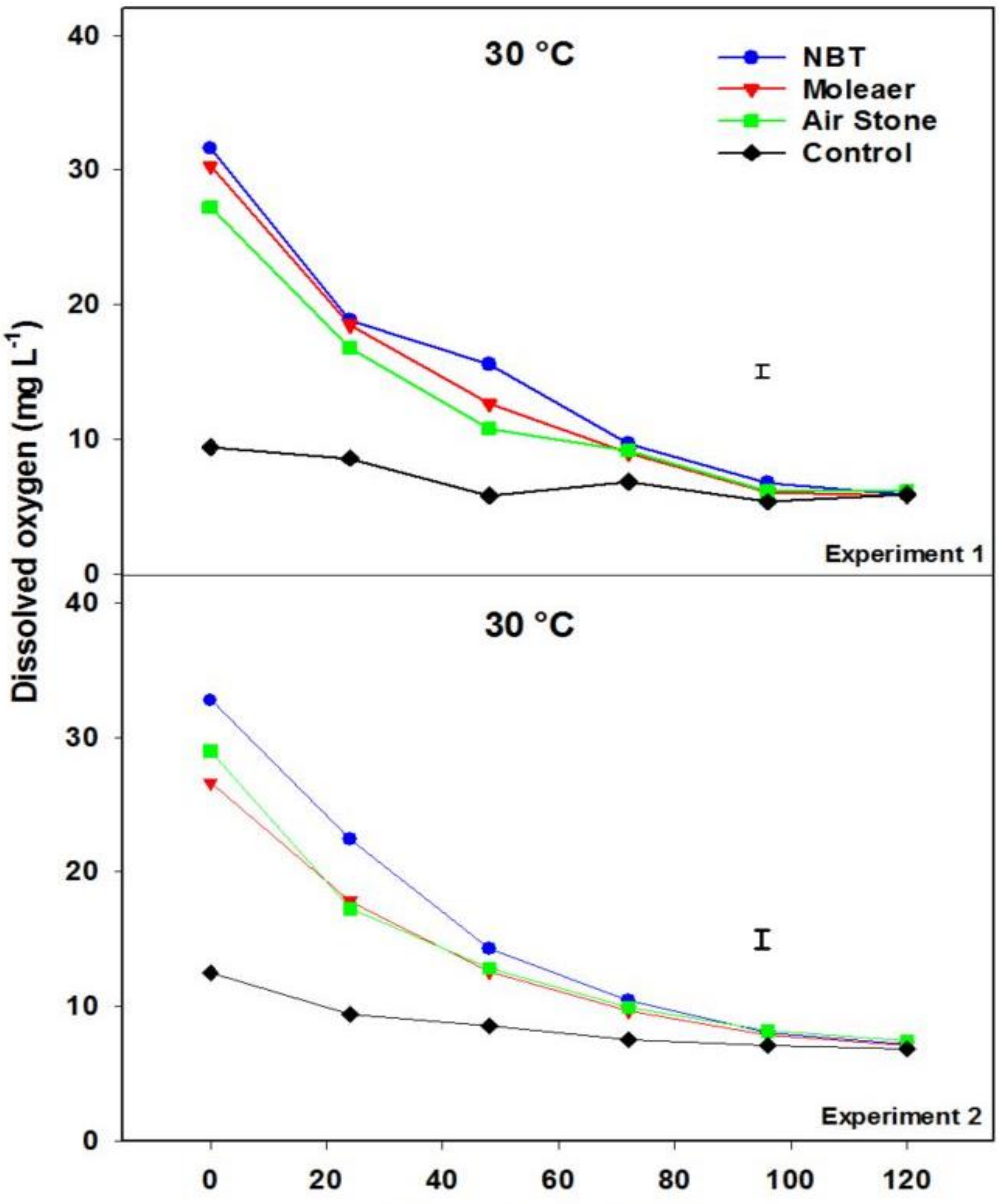

Figure 2.2. Effect of Oxygenation Method × Time on dissolved oxygen retention of three methods of water oxygenation and an untreated control at 30 °C. Error bar represents the least significant difference for comparing means (α = 0.05).

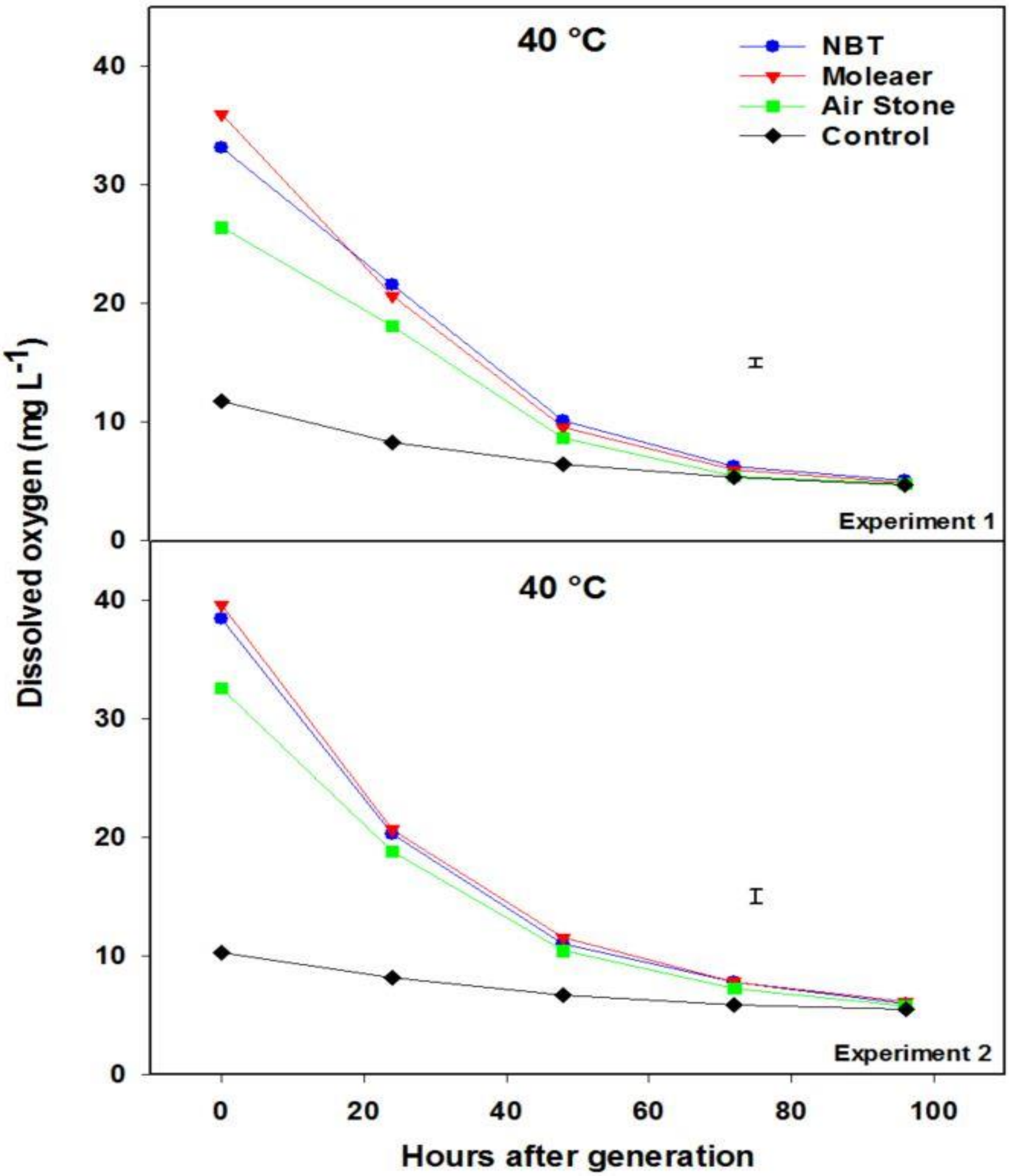

Figure 2.3. Effect of Oxygenation Method × Time on dissolved oxygen retention of three methods of water oxygenation and an untreated control at 40 °C. Error bar represents the least significant difference for comparing means ($\alpha = 0.05$).

Chapter 3. Effect of irrigation with nanobubble-oxygenated water sources on the growth of creeping bentgrass (*Agrostis stolonifera* L.) in a sand-based root zone

Abstract

Across much of the world, cultural and environmental factors can place creeping bentgrass (*Agrostis stolonifera* L.) under extreme stress during the summer months. This stress, coupled with the growth adaptation of bentgrass, can result in shallow, poorly rooted stands of turf. In an effort to enhance root zone characteristics and rooting of creeping bentgrass, golf course superintendents use various methods of increasing soil oxygen in putting green root zones, such as core aerification, solid-tine aerification, and sand topdressing. Another potential method of delivering additional oxygen to the soil could be irrigation with nanobubble-oxygenated water. Nanobubbles (NBs) are sub-micron, stable cavities of gas, typically between 200-700 nm. The properties of NBs allow for high gas dissolution rates in water. Irrigating with NB-oxygenated water sources may promote increased rooting of creeping bentgrass putting greens during high-temperature periods and lead to a healthier, more vigorous playing surface. The objectives of this study were to compare long-term irrigation effects with NB-oxygenated water sources to untreated water sources on creeping bentgrass putting green plant health characteristics, as well as soil oxygen and organic matter content. A three-year field study was conducted during the summers of 2019-2021. Treatments included NB-oxygenated potable water and irrigation pond water, oxygenated using a membrane-based NB injection system, and untreated potable and irrigation pond water. Long-term irrigation of creeping bentgrass with NB-oxygenated water did not enhance the measured plant health characteristics. In 2019, NB-oxygenated water increased the 15-cm soil depth daily mean partial pressure of oxygen from 17.48 kPa to 18.21 kPa corresponding to the control and NB-oxygenated water, respectively.

However, irrigation source did not affect soil oxygen levels in the other years of the trial. NB-oxygenation of irrigation water remains an excellent means of efficiently oxygenating large volumes of water. However, plant health benefits from irrigation of creeping bentgrass in a sand-based root zone with NB-oxygenated irrigation water were not observed over a 3-year trial period. Future research investigating irrigation of creeping bentgrass and other agricultural and horticultural crops cultivated in more finely textured root zones than that of a sand-based golf course putting green is warranted, as soil oxygen deficiency would be more likely in soils with a smaller percentage of macropores.

Introduction

Creeping bentgrass management

Creeping bentgrass (*Agrostis stolonifera* L.) is a popular and widely used turfgrass for golf course putting green surfaces due to its fine texture and tolerance of low mowing heights. Creeping bentgrass is a perennial, cool-season turfgrass best adapted for use in cool, humid environments. Primarily due to climate, maintenance of creeping bentgrass putting greens is difficult in the southern United States and much of the transition zone, a geographic region that spans from Oklahoma to the Mid-Atlantic states. This zone is referred to as the transition zone because it is a region where both cool- and warm-season grasses are grown, but it is not well suited for either type of grass. In the transition-zone region of Arkansas, cultural practices and environmental factors can place bentgrass under extreme stress during the summer period, resulting in shallow, poorly rooted stands of turf (Carrow, 1996; Fry & Huang, 2004).

In the summertime, drought stress of creeping bentgrass putting greens is often mitigated through daily or frequent irrigation. Irrigation and natural rainfall events can result in temporal saturation or waterlogging, especially on poorly drained putting greens (Jiang & Wang, 2006).

Madison and Hagan (1962) reported a reduction in the root mass of a Kentucky bluegrass (*Poa pratensis* L. 'Merion') stand attributed to frequent irrigation combined with low mowing heights. Waterlogging and the subsequent oxygen deficiency it causes have been shown to negatively impact creeping bentgrass growth (Huang et al., 1998; Jiang & Wang, 2006; Wang & Jiang, 2007). As soil oxygen becomes limiting, root cell metabolism will shift to fermentation, as mitochondrial respiration is compromised. Fermentation can result in an accumulation of toxic ethanol in the cells of roots, which may ultimately lead to root cell death (Pezeshki & DeLaune, 2012). Low-oxygen stress to turf may be compounded by increased demand for soil oxygen as respiration rates of plants and soil micro- and macro-organisms increase due to high temperatures and humidity in the summer months (Raich & Schlesinger, 1992).

Sand-based putting greens

Due to their resistance to compaction and rapid drainage potential, sandy soils are preferred for use as golf course putting green root zones (Lunt, 1956). One popular method of putting green construction, the United States Golf Association's recommendations for a method of putting green construction, requires that at least 60% of the putting green root zone be composed of medium to coarse textured sands, with corresponding particle sizes ranging from 0.25 to 1.0 mm in diameter (U.S. Golf Association, 2018). Even with the use of sand-based putting green root zones, reducing compaction and increasing the aeration of putting green soils is a common concern for golf course superintendents. Golf course superintendents use various methods to increase soil oxygen in putting green root zones, such as core aerification, solid-tine aerification, and sand topdressing. Other less commonly employed methods of increasing soil oxygen include direct injection of pressurized air into the soil (Air2G2, Foley Company, Prescott, WI), direct injection of water into the soil profile (HydroJect, The Toro Company, Bloomington, MN), and the installation of subsurface systems with root zone aeration, moisture control, and temperature moderating capabilities (SubAir, SubAir Systems LLC, Graniteville, SC). Regardless of the soil aeration method chosen, the costs and inconveniences associated with the previously mentioned methods make alternative, less disruptive aeration methods worthy of exploration.

Another component of creeping bentgrass putting green management that may affect soil oxygen levels is organic matter accumulation in the upper root zone (Carrow, 2004). Typically, golf course superintendents manage the accumulation of organic matter using a combination of methods such as physical removal by hollow-tine core cultivation or vertical mowing, dilution of organic matter by sand topdressing, and reducing organic matter accumulation by reducing fertilizer use to slow plant growth (Glasgow et al., 2005). If the organic matter content of a root zone is allowed to increase continually, macropores can become blocked, affecting root zone physical properties such as hydraulic conductivity, oxygen diffusion, and water retention (Adams & Saxon, 1979).

Oxygenating irrigation water

Kurtz and Kneebone (1980) investigated the effects of aeration and temperature on root growth characteristics of nine species of creeping bentgrass. Cut stolons were grown in bottles of tap water at 36, 38, and 40 °C. Bottles were either non-aerated or aerated by bubbling air from tubes attached to a small aquarium pump into the water. Aeration significantly increased rooting at all temperatures, with more pronounced differences observed at 40 °C. Sloan and Engelke (2005) examined the effects of continuous irrigation with ozonated and aerated water on creeping bentgrass growth and the physical and chemical properties of a sand-based root zone mix. Water was treated with an Oxion™ ozone generator (Thunderstorm Technology, Flowermound, TX), which passed ambient air through high-voltage corona discharge columns before introducing the air into the irrigation water. The aerated treatment was generated using the same Oxion^{TM} generator, but the charge on the corona discharge columns was reduced to zero. The dissolved oxygen (DO) content of the ozonated and aerated water ranged from 12-14 mg L^{-1}, while the untreated water had a DO content of 6-8 mg L^{-1}. The authors showed a short-term increase in creeping bentgrass clipping weight and chlorophyll content, attributed to increased soil nutrients from the ozone-facilitated oxidation of organic matter in the soil profile. The authors suggested that the effects may have been more pronounced in root zones suffering from low oxygen stress and stated that the beneficial effects from ozonated water became negligible after continuous application for an extended period. Guertal (2002) investigated the effects of oxygenated water on soil oxygen, root growth, and visual quality of a sand-based creeping bentgrass putting green. Two oxygenation methods included water oxygenated using a commercial oxygenator and water treated with hydrogen peroxide. The data suggested no increase in root growth, soil oxygen, or visual quality of the sand-based creeping bentgrass putting green. In a non-turf system, irrigating *Calibrachoa* × *hybrida* 'Aloha Kona Dark Red' and *Lobelia erinus* 'Bella Aqua' propagated in a porous, peat-based substrate and irrigated with oxygenated water did not enhance root or plant growth (Yafuso and Fisher, 2017). An additional method of water oxygenation that warrants further investigation for use in the irrigation of agricultural and horticultural crops is oxygenating water using nanobubble (NB) injection systems.

Nanobubbles

Nanobubbles (NBs) are gas-filled cavities in a liquid that are found dispersed within solution (bulk NBs) or at the interface between a liquid medium and a solid substrate (surface

NBs) (Wang et al., 2017). Although some disparity exists concerning the nomenclature and size of NBs, Temesgen et al. (2017) and Alheshibri et al. (2016) propose using the term NB to describe bubbles with a diameter of less than one μm. Early evidence of the presence of bubbles less than one μm in diameter was reported in seawater by Johnson and Cooke (1981). Since then, most studies involving NBs have focused on NB generation, detection, and characterization (Temesgen et al., 2017).

Nanobubbles have a large surface area per unit volume due to their small size, with a corresponding concentration as high as one hundred million to ten trillion bubbles per milliliter of liquid (Atkinson et al., 2019). Nanobubbles allow for high gas dissolution rates in liquids due to greater internal pressure in the bubble than their environment and high stagnation in the liquid phase (Ushikubo et al., 2010). Some studies also suggest that NBs exhibit a long residence time in solution owing to a negatively charged surface (zeta potential). This surface charge prohibits the coalescence of bubbles, which is characteristic of larger bubbles, which coalesce and rise to the surface (Ushikubo et al., 2010, Takahashi et al., 2007). Nanobubbles have been reported to remain in an aqueous solution for weeks and even months (Azevedo et al., 2016; Duval et al., 2012), while other studies have reported a rapid loss of oxygen in NB-oxygenated water (See Chapter 2; Langenfeld and Bugbee, 2021).

Nanobubbles are created using various methods, including cavitation, electrolysis, and membranes. According to Phan et al. (2020), the cavitation method of NB formation results in the formation of cavities when the homogenous liquid phase undergoes a phase change due to a sudden reduction of pressure below a particular critical value. The sudden reduction in pressure can be achieved through different methods relating to a fluid flow or an acoustic field, referred to as hydrodynamic and acoustic cavitation, respectively. Zhu et al. (2016) state that the creation of

NBs through the electrolysis of water involves the decomposition of water into hydrogen and oxygen gases caused by an electric potential. If the concentration of the produced gas reaches the supersaturation level in the anodic and cathodic streams of the bulk water, NBs can be generated. Membranes create NBs by acting as the medium for liquid and gas dispersion, where the gas phase is pressed through the pores of the applied membrane into a flowing aqueous phase (Phan et al., 2020). Nanobubble generation is a complex physiochemical process that depends on many factors, including temperature, applied pressure, electrolyte concentration, concentration and type of dissolved gas in solution, as well as concentration and type of surfactant (Phan et al., 2020). Several gases are commonly used to create NBs, including oxygen, air, ozone, carbon dioxide, and nitrogen, each utilized for their specific application (Phan et al., 2020).

Nanobubble irrigation

Horticultural applications for NBs have also been investigated. Ebina et al. (2013) compared the effects of air-NB treated water to untreated water on the growth of hydroponically cultured *Brassica campestris*. After four weeks, plants grown in air-NB-treated water had greater height, leaf length, and aerial fresh weight than untreated water. Wu et al. (2019) investigated NB-oxygenation to improve the mineralization of organic fertilizer and enhance crop growth in organic tomato production. The authors compared NB oxygenation to traditional pump-aerated oxygenation and a control group through soil incubation and soil column experiments. They reported a tomato yield increase from the NB-oxygenated treatment group of 23% and an increase from the traditional pump-aerated method of 17%, compared with the control. Multiple studies have also reported that NBs promote faster seed growth and germination times (Liu et al., 2016a; Liu et al., 2016b; Liu et al., 2017). Wang et al. (2021) reported enhanced nutrient absorption, a 25% reduction in fertilizer use, and increased yield when irrigating rice with air-NB water. Although promising, the effects of NB-oxygenated irrigation water have not yet been investigated on plants grown in more porous media, such as a sand-based putting green root zone (U.S. Golf Association, 2018). We hypothesize that compared to untreated water, long-term irrigation with NB-oxygenated water will increase the root zone oxygen concentration, increase root and shoot growth, and reduce the total organic matter content of an experimental sand-based creeping bentgrass putting green.

Materials and Methods

Experimental area

A three-year field study was conducted at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR (36.09 °N, 94.17 °W) to determine the effect of NB-oxygenated irrigation water on the growth of a sand-based creeping bentgrass putting green. Research was conducted from May through September during the summers of 2019-2021. Research was conducted on a mature, sand-based (U.S. Golf Association, 2004) creeping bentgrass (cv. Pure Distinction) putting green. Mowing was performed six days week^{-1} from March through October at a 3.0-mm bench setting height of cut using a Toro Greensmaster 3250-D (The Toro Co. Bloomington, MN). From May through October, nitrogen (N) was applied bi-weekly at a rate of 13 kg ha^{-1}, alternating between water-soluble urea (Thrive 46-0-0, Mears Fertilizer Inc., El Dorado, KS) and Contec DG granular fertilizer (18-9-18, The Andersons, Maumi, OH). Phosphorous, potassium, and micronutrients were applied according to annual soil testing recommendations (Espinoza et al., 2006). Sand topdressing was applied at 0.3 L m^{-2} at two-week intervals from March through October. Core aerification and use of plant growth regulators were conducted according to typical putting green management practices for the region.

Treatments

In 2019, a proprietary membrane-based NB injection system (Nano Bubble Technologies, Sydney, AU) was used to oxygenate potable water from the Beaver Water District (Lowell, AR) (Appendix A-2). Nanobubble-oxygenated water was cycled through the NB injection system delivering 1.5 L min^{-1} of industrial-grade oxygen to a water volume of 378 L, cycling for a period of two hours at a rate of 26 L min^{-1}. In 2019, NB-oxygenated water was compared to potable tap water.

In 2020 and 2021, a second water source was added to the study. The membrane-based nanobubble injection system (Nano Bubble Technologies, Sydney, AU) was used to oxygenate both potable water from the Beaver Water District (Lowell, AR) and irrigation pond water from the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR (Appendix A-3, A-4). The two main effects of water source (Potable or Pond) and NB-oxygenation (NB-oxygenated or Untreated) were arranged in a 2 × 2 factorial totaling four treatments that included the following:

Potable water treatments (Potable):

- Oxygenated using the NB injection system (NBT - POT)
- Untreated (POT)

Irrigation-pond water treatments (Pond):

- Oxygenated using the NB injection system (NBT - PND)
- Untreated (PND)

In all three seasons, each water treatment was transferred to a 98-L, 12-volt sprayer system (NorthStar ATV 12v Spot Sprayer, Clayton Engineering, Brendale, QLD, AU) for transport and final delivery of the water to the putting green from a drenching nozzle (Cool Shot Plus, Weathermatic, Garland, TX) delivering 20 L min^{-1}. In 2019, irrigation treatments were applied 3x $week^{-1}$ to replace 140% reference evapotranspiration (ET) to deliver maximum dissolved oxygen to the root zone. Due to a lack of treatment effects in 2019 and to simulate a real-world irrigation strategy more closely, in 2020 and 2021, total ET replacement was adjusted, and treatments were applied 3x $week^{-1}$ to replace 100% ET. Irrigation was omitted when precipitation totals were greater than replacement ET. Individual plots measured 1.2- by 1.2-m with .5-m alleys to minimize lateral movement of varying water sources in the soil profile. Monthly water quality testing was conducted by the Arkansas Water Resources Center Water Quality Laboratory for potable and irrigation pond water.

Data collection

Soil oxygen levels, reported as the partial pressure of oxygen (PPSO), were continuously monitored in two of the four replications at a 15-cm soil depth in 2019 and 2020 and a 7-cm soil depth in 2021, using eight permanently installed oxygen sensors (Apogee SO-110 Soil Response Thermistor Reference Oxygen Sensors, Apogee Instruments, Logan, UT). Sensors were connected to a data logger equipped with a multiplexer (CR1000 and AM16/32b, Campbell Scientific, Logan, UT), and readings were recorded every 15 minutes. Daily average soil-oxygen levels were determined and used for statistical comparison. Green turfgrass coverage (GTC) and dark green color index (DGCI) were evaluated weekly using digital image analysis (DIA) (Karcher & Richardson, 2003; Richardson et al., 2001). Images were obtained using a digital camera (Canon PowerShot G12, Canon Inc., Melville, NY) mounted to a 0.9- by 0.9-m metal box equipped with four light bulbs, providing a consistent light source to collect comparable images. Images collected were then analyzed for GTC and DGCI using TurfAnalyzer (Karcher et al., 2017). For GTC, green pixels were selected based on a hue range of 45 to 125 and a saturation range from 10 to 100. The total number of green pixels was divided by the total number of pixels present in the image to calculate the percent green turfgrass coverage present in the image. Clipping yield (g m^{-2}) was collected every second week by mowing the entire area of each experimental plot using a reel-type mower and bucket. Clippings were oven dried for at least 72 hours at 80 °C and weighed. Prior to weighing, sand and debris were separated from turfgrass clippings via the vibrating pan method described by Kreuser et al. (2011). Collected clippings were sent to the University of Arkansas Agricultural Diagnostic Laboratory and analyzed for total-N by combustion (Campbell & Plank, 1992) and nitrate-N using the modified Cataldo method (Cataldo et al., 2008). Root samples were collected monthly using a soil profiler (MPS1-S, Turf-Tec International, Tallahassee, FL) in two random locations within each plot. Roots were washed and analyzed for total surface area, total length, average diameter, and total volume using image scanning analysis (WinRhizo, Regent Instruments, Quebec City, Canada). In 2020 and 2021, the total organic matter content (TOMC) for each treatment was determined monthly by weight loss on ignition. Three soil cores measuring 2.5 cm in diameter were extracted from each experimental unit to a depth of 10 cm. Cores were divided into sections measuring 0-5 cm depth and 5-10 cm depth. Leaf tissue was not removed from the soil cores to reduce sample preparation variability. Cores were oven dried at 80 °C for 72 hr. Cores were ground, weighed, and placed in a muffle furnace at 440 °C for 5 hr. Cores were reweighed, and total weight loss was used to determine the percentage of organic matter present in each sample.

Experimental design and statistical analysis

Treatments were replicated four times in a randomized complete block design. All data were analyzed separately each year due to inconsistencies in evaluation dates and methodologies between years. In order to best estimate cumulative treatment effects throughout the summer, repeated measures analysis of variance ($P < 0.05$) was used to evaluate the effects of water source, NB-oxygenation, and their interaction on soil oxygen content, GTC, DGCI, root morphology, clipping yield, clipping N content, and TOMC using PROC MIXED in SAS v. 9.4., with date included in the analysis. Where appropriate, treatment means were separated using Fisher's least significant difference test ($\alpha = 0.05$). Slicing was performed to determine specific dates when significant treatment effects were present.

Results

Soil oxygen

Nanobubble-oxygenated irrigation water inconsistently affected the PPSO over three seasons (Table 3.1). In 2019, NB-oxygenated water increased the daily mean PPSO from 17.48 kPa in untreated water to 18.21 kPa in NB-oxygenated water. In 2020, NB-oxygenated irrigation water reduced the PPSO (19.7 kPa) compared to untreated water sources (20.3 kPa). In 2021, no effect or interaction significantly affected PPSO.

Digital image analysis

Irrigating with NB-oxygenated water sources never enhanced GTC or DGCI. Date was the only treatment that resulted in significant differences in GTC, as NB-oxygenation failed to increase GTC in any season of research (Table 3.2). Green turfgrass coverage ranged from 92 to 99% with a mean of 98%. Dark green color index was inconsistently affected by NB-oxygenation of irrigation water over three seasons of research (Table 3.2). In 2019, DGCI was only affected by Date (Table 3.2). In 2020, the main effect of NB-Oxygenation reduced the DGCI of NB-oxygenated water sources (0.4332) compared to untreated sources (0.4405) (Fig. 3.1). The Water Source × Date interaction in 2020 resulted in a significant increase in DGCI for

Potable treatments (0.6724) compared to Pond treatments (0.6501) on one sampling date (Fig. 3.2). In 2021, DGCI was affected by the highest order interaction of Water Source × NB-Oxygenation × Date (Table 3.2). This interaction resulted in a significantly greater DGCI in the PND treatment compared to other treatments on multiple dates (Fig. 3.3). The greater DGCI values observed in the PND treatment can be attributed to a greening effect caused by a numerically greater iron content in the pond water source compared to the potable water source (Table A4). This greening effect was less pronounced in the NBT-PND treatment due to the NB oxygenation process resulting in reduced iron solubility in the oxygenated water.

Clippings

Nanobubble-oxygenation of irrigation water did not increase clipping yield, clipping total-N, or clipping nitrate-N in any season of research. Clipping yield was unaffected by any treatment other than Date in 2020 and 2021 (Table 3.3). Clipping yields varied greatly with a range of 0.5 to 19.0 g m^{-2}, with a mean clipping yield of 6.53 g m^{-2}. Clipping total-N was only affected by the Water Source × Date interaction in 2021 (Table 3.3). The interaction of Water Source × Date resulted in greater clipping total-N in Potable water treatments compared to Pond water treatments on one date in 2021 (Fig. 3.4). Clipping nitrate-N was unaffected by treatment other than Date in both seasons (Table 3.3). Mean clipping nitrate-N was determined to be 517 mg kg^{-1}

Root growth

Nanobubble-oxygenation of irrigation water did not affect measured root morphological characteristics in any season. Root morphological characteristics determined by WinRhizo image scanning software were unaffected by any treatment or interaction other than by Date (Tables 3.4, 3.5). WinRhizo determined that total root length ranged from 543 to 3627 cm, with a mean of 1651 cm. Total root surface area ranged from 64 to 547 cm^2 with a mean of 274 cm^2. Root average diameter ranged from 0.23 to 0.58 mm with a mean of 0.42 mm. Total root volume ranged from 0.64 to 8 cm^3 with a mean of 2.76 cm^3.

Total soil organic matter

Nanobubble-oxygenation of irrigation water did not affect TOMC in any season. Throughout the trial, TOMC was only affected by Date, Depth, and their interaction (Table 3.6). As expected, TOMC decreased with increasing root zone depth, and varied naturally by sampling date. Total organic matter content ranged from 3.7 to 8.4% at the 0-5 cm depth and 0.4 to 2% at the 5-10 cm depth, with a mean of 5.4% and 1.25%, respectively.

Discussion

Oxygen diffusion rate (ODR) is a common metric used to express the soil aeration status of turfgrass root zones but does not provide an absolute concentration of oxygen present in the soil (Neira et al., 2015; Sojka & Scott, 2000). Research from Guertal (2002) demonstrated that irrigation with oxygenated water never affected the ODR of a sand-based creeping bentgrass putting green. Although the soil ODR in our research was not measured directly, the partial pressure of soil oxygen (PPSO) was used to indicate the soil oxygen status. The PPSO was only affected by oxygenated water in one season of research when a statistically significant increase in the mean daily PPSO was detected from irrigation with NB-oxygenated water. The increase in the PPSO was less than one kPa and did not improve turfgrass quality or other performance metrics.

Many questions remain regarding the fate of DO in irrigation water if no effect on the ODR was detected in Guertal's research and minimal effect on PPSO was observed in the present study. Determining the fate of the DO contained in the NB-irrigation water could help explain the lack of detectable treatment effects on the PPSO. The DO concentration of the irrigation water used in this research was monitored at multiple stages of the oxygenation and irrigation process, including in the NB-oxygenation circulation tank, in the irrigation application sprayer tank, and finally, at the surface of the turf after passing through the sprayer pump and hose nozzle. Dissolved oxygen loss from irrigation water was not observed between the circulation tank and the sprayer tank. However, during irrigation events, as NB-oxygenated water passed through the irrigation pump and hose nozzle and made contact with the turf surface, an average of 55% of the initial DO concentration was lost from the NB-oxygenated water. Even so, the DO content of irrigation water caught on the putting green surface consistently measured more than 10 mg L^{-1} greater than the level of DO in untreated water.

Therefore, it makes sense that if the DO contained in the water was delivered to soil depths as low as 7 cm (shallowest sensor depth), the soil oxygen response thermistors would have detected differences in PPSO. The lack of consistently detectable differences in PPSO means that the oxygen contained in the NB-oxygenated treatments was subject to one or a combination of fates, including i) being consumed immediately by soil organisms and redox processes; ii) remaining in the root zone above a 7-cm depth; iii) gassing off into the atmosphere during infiltration; iv) not detectable by the soil oxygen sensors. According to Bhattarai et al. (2005), measuring the partial pressure of soil oxygen (PPSO) can be a challenging task due to the heterogeneity of soil. The influence of soil heterogeneity on PPSO measurements increases with decreasing sensor size and increasing spatial resolution. The size and number of sensors installed may help partially explain a lack of consistently detectable differences in PPSO.

Whether or not DO was delivered deep enough in the root zone to affect plant growth responses, it was apparent that the soil in our research never reached sufficient levels of hypoxia to induce reductions in plant health characteristics. The green turfgrass coverage of our experimental plots was never observed to be below 95% on any sampling date in all three years of research. If the DO did reach the roots of our experimental creeping bentgrass putting green, the lack of plant response to NB-oxygenated irrigation likely resulted from the presence of a sufficient oxygen supply in the soil for the entirety of the trial due to the large number of air-filled pores, typical of sand-based putting green root zones. A lack of plant response to oxygenated irrigation water was also observed by Yafuso and Fisher (2017), Bonachella et al. (2010), Ehret et al. (2010), Bonachella et al. (2005), and Guertal (2002), who all observed little, or no plant health benefits from irrigating with oxygenated water in course-textured or porous media.

Using either aerated or non-aerated water baths as a growing medium, Kurtz and Kneebone (1980) observed enhanced creeping bentgrass growth by cultivating bentgrass in aerated water. The authors attributed the increased bentgrass growth to greater DO concentrations present in the aerated water treatment. Enhanced growth was likely due to the water in their research acting as the soil medium, similar to hydroponic production, where plant roots harvest the DO present in the nutrient solution. In the Kurtz and Kneebone research, bentgrass roots had access only to the DO present in the irrigation water, resulting in increased plant growth at higher levels of DO. Our research differed in that bentgrass grew in a sand-based medium with a large percentage of air-filled soil pores, which likely provided adequate soil aeration throughout our research.

Research demonstrating the benefits of irrigating with oxygenated water in sand-based soils, such as a putting green built to USGA specifications, is not currently available. However, subsurface irrigation with oxygenated water has increased the yield and water use efficiency of potato (*Solanum* tuberosum) in a sandy clay loam (Shahein et al., 2014), increased corn yield in a water-logged vermiculite substrate (Lei et al., 2016), increased yield of cotton (*Gossypium hirsutum*), soybean (*Glycine max*), and zucchini (*Cucurbita pepo*) in saturated, heavy clay soil (Bhattarai et al., 2004), and increased pineapple (*Ananas comosas*) fresh biomass and dry matter weight in a loamy sand soil (Chen et al., 2011). The soil in our research was not fine-textured, did not remain waterlogged for extended periods, and was not irrigated using subsurface methods, three commonalities observed in research demonstrating increased plant growth from oxygenated irrigation water.

Organic matter was not affected by NB-oxygenation or irrigation water source in this study. Organic matter decomposition was likely not limited by oxygen availability in this specific experimental putting green. Sloan and Engelke (2005) observed a reduction of organic matter in their experimentally constructed sand-based creeping bentgrass growth columns due to irrigation with ozonated water. The authors attributed the increased organic matter oxidation to irrigation with ozonated water, a powerful oxidizer. Ozone was not used to oxygenate the irrigation water in our research, which may have resulted in the observed differences in organic matter reduction between our work and that of Sloan and Engelke.

Conclusions

No benefit to sand-based creeping bentgrass putting green health was observed from irrigation with NB-oxygenated water sources. Soil oxygen and plant health characteristics measured in this research were rarely affected by NB-oxygenated irrigation water. Nanobubble-oxygenated irrigation water increased the PPSO compared to untreated water in one season, but this was not observed in any other season. During irrigation events, greater than 50% of total DO was lost from NB-oxygenated water due to the turbulence and subsequent increase in the surface area of the water after passing through an irrigation nozzle and contacting the putting green. Results from this trial do not support our hypothesis that long-term irrigation of a creeping bentgrass putting green with NB-oxygenated water would increase root zone oxygen concentration, increase root and shoot growth compared to untreated irrigation water sources, or reduce organic matter content of an experimental sand-based creeping bentgrass putting green. Before investing in an NB injection system, end-users should seek out research that indicates NB-oxygenation of irrigation water can be beneficial in their specific system or circumstance.

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Table 3.1. Analysis of variance testing the main effects of irrigation water source (Potable or Pond), nanobubble-oxygenation (NB-Oxygenated or Untreated), Date and their interactions on the partial pressure of soil oxygen in a sand-based creeping bentgrass putting green irrigated with nanobubble-oxygenated water during the summers of 2019-21.

Effect	**Soil oxygen (kPa)**		
	2019	2020	2021
	------------------- *P* > *F* -------------------		
Water Source		NS	NS
NB-Oxygenation	0.0256	0.0313	NS
Water Source × NB-Oxygenation		NS	NS
Date	<.0001	<.0001	<.0001
Water Source × Date		NS	NS
NB-Oxygenation × Date	NS[a]	NS	NS
Water Source × NB-Oxygenation × Date		NS	NS

[a] NS, nonsignificant at the 0.05 probability level.

Table 3.2. Analysis of variance testing the main effects of irrigation water source (Potable or Pond), nanobubble-oxygenation (NB-Oxygenated or Untreated), Date, and their interactions on the dark green color index (DGCI) and green turfgrass coverage (GTC) of a sand-based creeping bentgrass putting green irrigated with nanobubble-oxygenated water during the summers of 2019-21.

Effect	**DGCI**	**GTC**	**DGCI**	**GTC**	**DGCI**	**GTC**
	2019		2020		2021	
	---------- *P* > *F* ----------					
Water Source			NS	NS	0.0226	NS
NB-Oxygenation	NS[a]	NS	0.0415	NS	NS	NS
Water Source × NB-Oxygenation			NS	NS	NS	NS
Date	<.0001	NS	<.0001	<.0001	<.0001	<.0001
Water Source × Date			0.0385	NS	0.0101	NS
NB-Oxygenation × Date	NS	NS	NS	NS	NS	NS
Water Source × NB-Oxygenation × Date			NS	NS	0.0005	NS

[a] NS, nonsignificant at the 0.05 probability level.

Table 3.3. Analysis of variance testing the main effects of irrigation water source (Potable or Pond), nanobubble-oxygenation (NB-Oxygenated or Untreated), Date, and their interactions on clipping dry weight, clipping total nitrogen content, and clipping nitrate nitrogen content of a sand-based creeping bentgrass putting green irrigated with nanobubble-oxygenated water during the summers of 2020 and 2021.

Effect	**Clipping dry weight (g)**			**Total nitrogen (ppm)**		**Nitrate content (%)**	
	2019	2020	2021	2020	2021	2020	2021
	-- *P* > *F* --						
Water Source		NS	NS	NS	NS	NS	0.0334
NB-Oxygenation	NS[a]	NS	NS	NS	NS	NS	NS
Water Source × NB-Oxygenation		NS	NS	NS	NS	NS	NS
Date	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Water Source × Date		NS	NS	NS	0.0290	NS	NS
NB-Oxygenation × Date	NS	NS	NS	NS	NS	NS	NS
Water Source × NB-Oxygenation × Date		NS	NS	NS	NS	NS	NS

[a] NS, Nonsignificant at the 0.05 probability level.

Table 3.4. Analysis of variance testing the main effects of irrigation water source (Potable or Pond), nanobubble-oxygenation (NB-Oxygenated or Untreated), Date, and their interactions on the root mass of a sand-based creeping bentgrass putting green irrigated with nanobubble-oxygenated irrigation water during the summers of 2020-21.

Effect	**Weight (g)**	
	2020	2021
Water Source	------------------ *P* > *F* ---------------	
NB-Oxygenation	NS[a]	NS
Water Source × NB-Oxygenation	NS	NS
Date	<.0001	<.0001
Water Source × Date	NS	NS
NB-Oxygenation × Date	NS	NS
Water Source × NB-Oxygenation × Date	NS	NS

[a] NS, nonsignificant at the 0.05 treatment level.

Table 3.5. Analysis of variance testing the main effects of irrigation water source (Potable or Pond), nanobubble-oxygenation (NB-Oxygenated or Untreated), Date, and their interactions on root growth properties of a sand-based creeping bentgrass putting green irrigated with nanobubble-oxygenated irrigation water during the summers of 2020 and 2021.

Effect	**Average diameter (mm)**			**Length (cm)**			**Surface area (cm^2)**			**Volume (cm^3)**		
	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021
	-- *P* > *F* --											
Water Source		NS	NS		NS	NS		NS	NS		NS	NS
NB-Oxygenation	NS[a]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Water × NB-Oxy		NS	NS		NS	NS		NS	NS		NS	NS
Date	0.283	<.0001	0.0650	NS	<.0001	<.0001	NS	<.0001	<.0001	<.0001	<.0001	<.0001
Water × Date		0.0163	NS		NS	NS		NS	NS		0.0419	NS
NB Oxy × Date	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Water × NB × Date		NS	NS		NS	NS		NS	NS		NS	NS

[a] NS, Nonsignificant at the 0.05 probability level.

Table 3.6. Analysis of variance testing the main effects of irrigation water source (Potable or Pond), nanobubble-oxygenation (NB-Oxygenated or Untreated), Depth, Date, and their interactions on the soil organic matter content of a sand-based creeping bentgrass putting green irrigated with nanobubble-oxygenated water during the summers of 2020 and 2021.

Effect	**Soil organic matter (%)**	
	2020	2021
	--------- *P* > *F* --------	
Water Source	NS[a]	NS
NB-Oxygenation	NS	NS
Water Source × NB-Oxygenation	NS	NS
Depth	<.0001	<.0001
Water Source × Depth	NS	NS
NB-Oxygenation × Depth	NS	NS
Water Source × NB-Oxygenation × Depth	NS	NS
Date	<.0001	<.0001
Water Source × Date	NS	NS
NB-Oxygenation × Date	NS	NS
Water Source × NB-Oxygenation × Date	NS	NS
Depth × Date	0.037	0.0074
Water Source × Depth × Date	NS	NS
NB-Oxygenation × Depth ×Date	NS	NS
Water Source × NB-Oxygenation × Depth × Date	NS	NS

[a] NS, Nonsignificant at the 0.05 probability level.

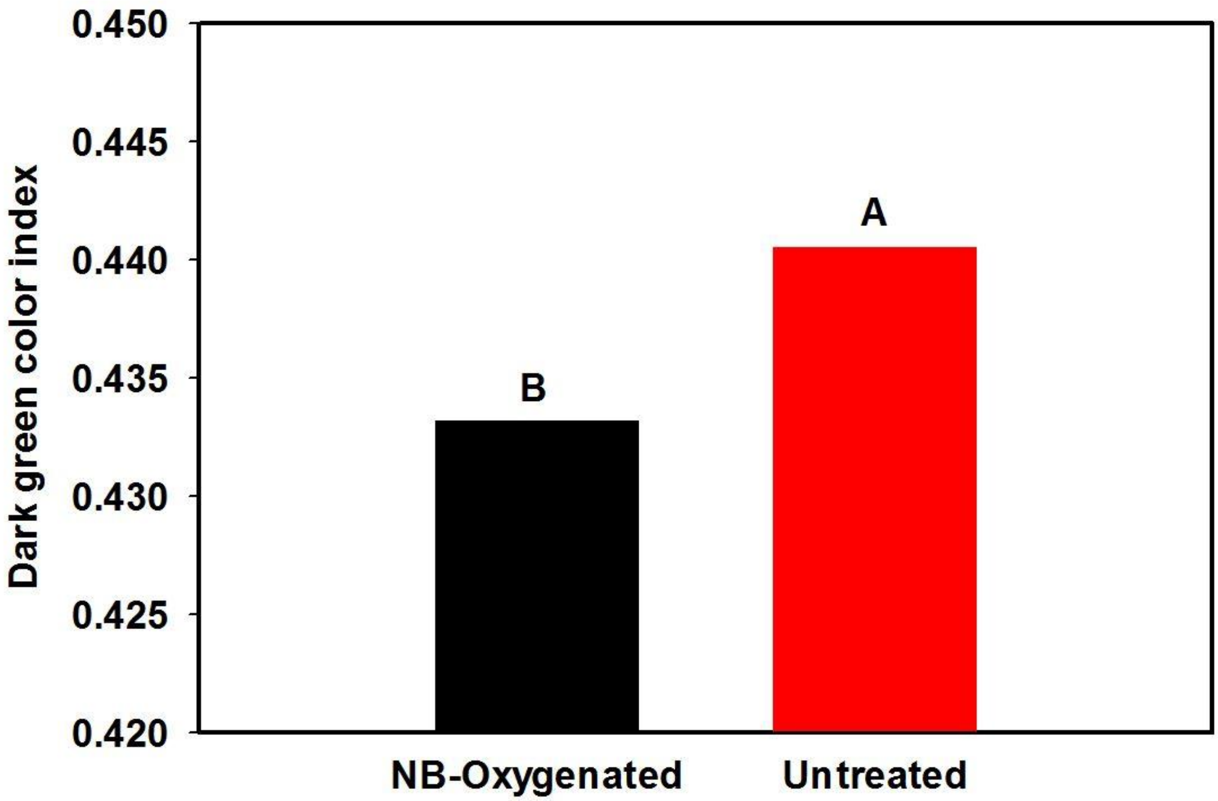

Figure 3.1. Effect of nanobubble-oxygenation of irrigation water sources on the dark green color index of a sand-based creeping bentgrass putting green during the summer of 2020 in Fayetteville, AR. Different letters indicate a significant difference between treatment means ($p < 0.05$).

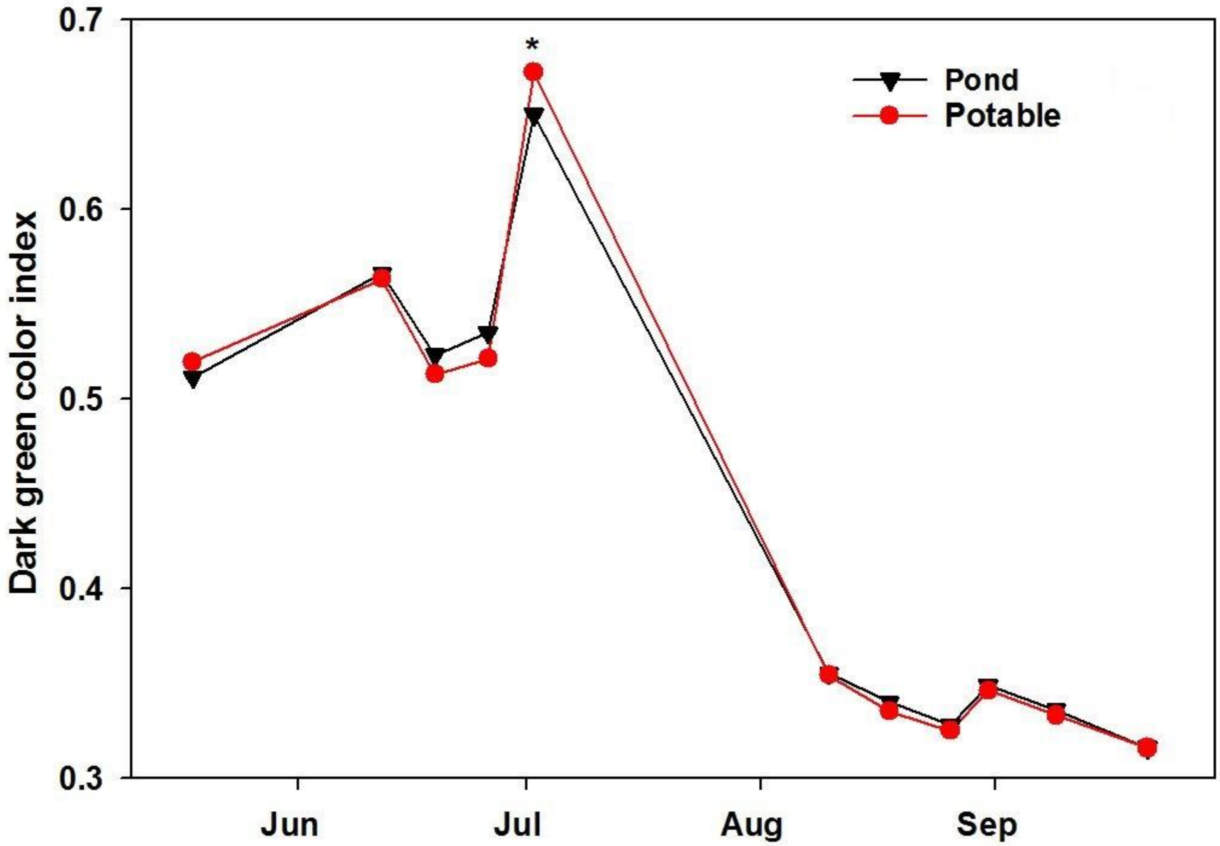

Figure 3.2. Effect of the Water Source × Date interaction on the dark green color index of a sand-based creeping bentgrass putting green during the summer of 2020 in Fayetteville, AR. Asterisk indicates date with a significant difference between treatment means ($p < 0.05$).

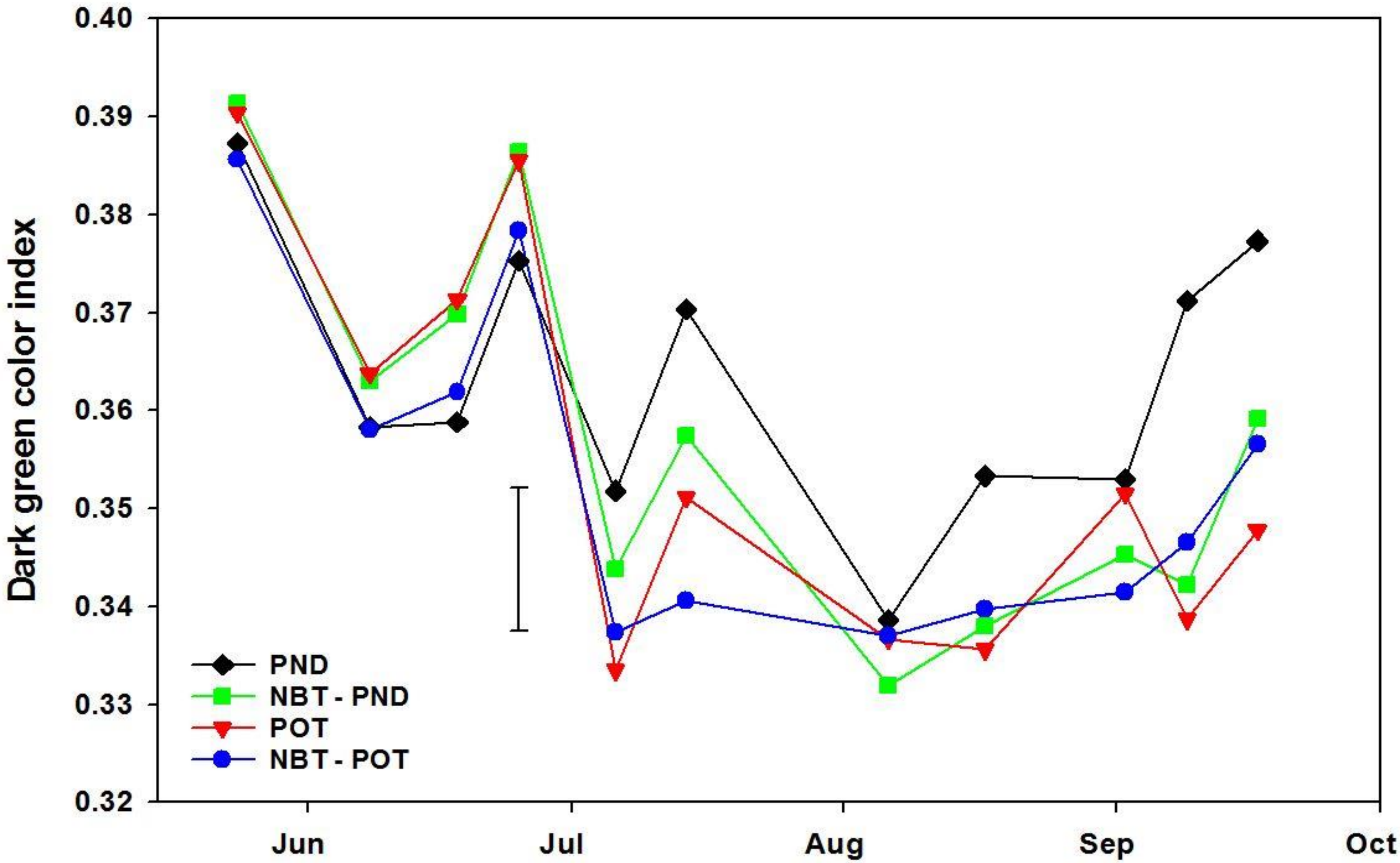

Figure 3.3. Effect of the Water Source [potable (POT) or pond (PND)] × NB-Oxygenation [nanobubble oxygenated (NBT) or untreated] × Date interaction on the dark green color index of a sand-based creeping bentgrass putting green during the summer of 2021 in Fayetteville, AR. Error bar represents the least significant difference for comparing means ($\alpha = 0.05$).

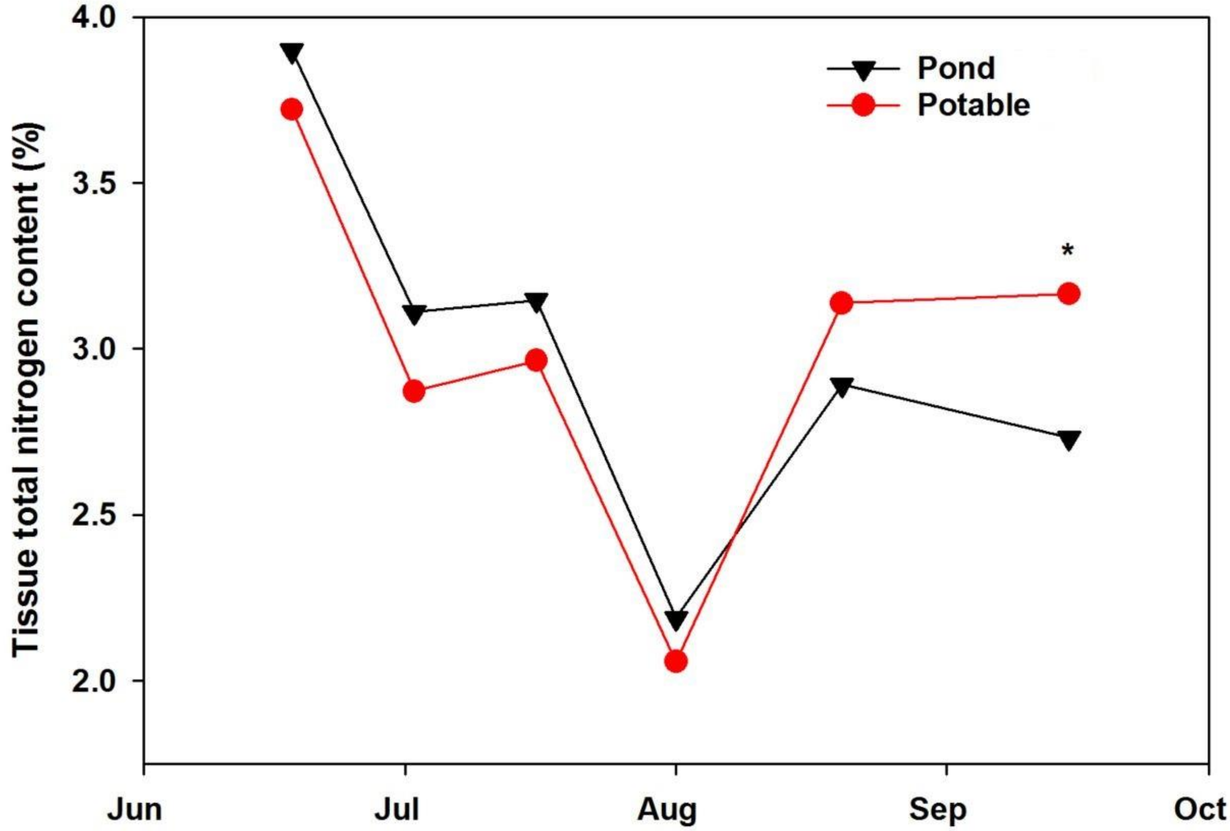

Figure 3.4. Effect of Water Source (Potable or Pond) × Date on the leaf tissue total nitrogen content of a sand-based creeping bentgrass putting green during the summer of 2021 in Fayetteville, AR. Asterisk indicates date with a significant difference between treatment means ($p < 0.05$).

Chapter 4. Effect of subsurface irrigation with nanobubble-oxygenated water sources on the growth of creeping bentgrass (*Agrostis stolonifera* L.) in a controlled environment

Abstract

Plant roots require access to oxygen within the soil to perform aerobic respiration. Dissolved oxygen (DO) in water can be delivered to soil and used for plant respiratory processes. Nanobubbles (NB) are sub-micron, stable cavities of gas with characteristics that allow for high gas dissolution rates in water. The use of overhead irrigation with NB-oxygenated water to deliver oxygen to the root zone of a sand-based creeping bentgrass (*Agrostis stolonifera* L.) putting green has been investigated in the past with no effect on plant growth. Nearly 55% of total DO was lost from NB-oxygenated water after passing through an irrigation nozzle and contacting the surface of the putting green when irrigating. Subsurface irrigation with NB-oxygenated water should reduce DO loss from irrigation water by reducing the amount of turbulent action to which the irrigation water is exposed. Thus, this research aimed to compare the effects of subsurface irrigation with NB-oxygenated water sources on creeping bentgrass plant health characteristics when grown in sand-based lysimeters. Lysimeters were constructed with a sand-based root zone conforming to United States Golf Association specifications. The study was a 2 × 2 factorial design with two water treatments (Potable or Pond) and two oxygen treatments (NB-Oxygenated or Untreated), arranged in a randomized complete block design with four replications. Lysimeters were irrigated 3x week^{-1} using subsurface irrigation. Digital image analysis was conducted weekly to monitor green turfgrass coverage and dark green color index. Leaf clippings were collected every other week and analyzed for total nitrogen (%) and nitrate-N (mg L^{-1}). Roots were analyzed for dry weight and growth characteristics. Subsurface irrigation with NB-oxygenated water did not affect measured creeping bentgrass plant health

characteristics. This research demonstrates no benefit from NB-oxygenation of irrigation water to creeping bentgrass plant health characteristics when grown in a sand-based root zone. Investigating the effects of subsurface irrigation with NB-oxygenated water in finer textured root zones may help elicit plant growth responses from increased DO in soil solution.

Introduction

Creeping bentgrass management

Creeping bentgrass (*Agrostis stolonifera* L.) is a popular and widely used turfgrass for golf course putting green surfaces due to its fine texture and tolerance of low mowing heights. Creeping bentgrass is a perennial, cool-season turfgrass best adapted for use in cool, humid environments. Primarily due to climate, maintenance of creeping bentgrass putting greens is difficult in the southern United States and much of the transition zone, a geographic region that spans from Oklahoma to the Mid-Atlantic states. This zone is referred to as the transition zone because it is a region where both cool- and warm-season grasses are grown, but it is not well suited for either type of grass. In the transition-zone region of Arkansas, cultural practices and environmental factors can place bentgrass under extreme stress during the summer period, resulting in shallow, poorly rooted stands of turf (Carrow, 1996; Fry & Huang, 2004).

In the summertime, drought stress of creeping bentgrass putting greens is often mitigated through daily or frequent irrigation. Irrigation and natural rainfall events can result in temporal soil saturation or waterlogging, especially on poorly drained putting greens (Jiang & Wang, 2006). Madison and Hagan (1962) reported a reduction in the root mass of a Kentucky bluegrass (*Poa pratensis* L. ‘Merion’) stand under frequent irrigation and low mowing heights. Waterlogging and the subsequent oxygen deficiency it causes have been shown to negatively impact creeping bentgrass growth (Huang et al., 1998; Jiang & Wang, 2006; Wang & Jiang, 2007). As soil oxygen becomes limiting, root cell metabolism will shift to fermentation, as mitochondrial respiration is compromised. Fermentation can result in an accumulation of toxic ethanol in the cells of roots, which may ultimately lead to root cell death (Pezeshki & DeLaune, 2012). Low-oxygen stress to turf may be compounded by increased demand for soil oxygen as respiration rates of plants and soil micro- and macro-organisms increase due to high temperatures and humidity in the summer months (Raich & Schlesinger, 1992).

Sand-based putting greens

Due to their resistance to compaction and rapid drainage potential, sandy soils are preferred for use as golf course putting green root zones (Lunt, 1956). One popular method of putting green construction, the United States Golf Association's recommendation for a method of putting green construction, requires that at least 60% of the putting green root zone be composed of medium to coarse textured sands, with corresponding particle sizes ranging from 0.25 to 1.0 mm in diameter (U.S. Golf Association, 2018). Although a USGA putting green has an inherently porous root zone, reducing compaction and increasing the aeration of putting green soils is a common concern for golf course superintendents. Golf course superintendents use various methods to increase soil oxygen in putting green root zones, such as core aerification, solid-tine aerification, and sand topdressing. Other less commonly employed methods of increasing soil oxygen include direct injection of pressurized air into the soil (Air2G2, Foley Company, Prescott, WI), direct injection of water into the soil profile (HydroJect, The Toro Company, Bloomington, MN), and the installation of subsurface aeration systems powered by a vacuum blower (SubAir, SubAir Systems LLC, Graniteville, SC). Regardless of the soil aeration method chosen, the costs and inconveniences associated with the previously mentioned methods make alternative, less disruptive aeration methods worthy of exploration.

Subsurface irrigation

Dissolved oxygen in irrigation water can also be delivered to the soil and used for soil respiratory processes. Enhancing the amount of DO in irrigation water has been attempted in the past to improve crop yield and water use efficiency, with variable results. Nonetheless, scientists have shown increased yields of 10-20% when crops were irrigated with oxygenated water (Du et al., 2018). Oxygation refers to the irrigation of crops with aerated water (containing increased dissolved oxygen levels). Water for oxygation is typically aerated through air injection using the Venturi principle, or hydrogen peroxide delivered to the soil, and the aerated water is commonly applied through subsurface drip irrigation (SDI) (Bhattarai et al., 2005). Pendergast et al. (2013) investigated the effects of oxygenated SDI on the growth of cotton (*Gossypium hirsutum* L.) in a vertosol over seven years and observed a 10% increase in cotton yield and a 7% increase in water-use efficiency. Bhattarai et al. (2004) increased the biomass of zucchini (*Cucurbita pepo*), soybean (*Glycine max*), and cotton in heavy clay soils using oxygenated SDI, attributing the increase to aeration-induced enhanced root function. Air-injected SDI has also increased bell pepper growth (*Capsicum annuum*) in sandy loam soil compared to the unaerated control (Goorahoo et al., 2002). Oxygation through SDI increased pineapple (*Ananas comosas*) fresh biomass and dry matter weight in a loamy sand soil (Chen et al., 2011). Previous research on oxygation has primarily been conducted with agricultural crops and focused on using SDI aeration by forced air injection. Newer technologies utilizing nanobubble-oxygenation could increase dissolved oxygen levels in treated water compared to standard water aeration methods.

Nanobubbles

Nanobubbles (NBs) are gas-filled cavities in a liquid that are found dispersed within solution (bulk NBs) or at the interface between a liquid medium and a solid substrate (surface

NBs) (Wang et al., 2017). Some disparity exists concerning the nomenclature and size of NBs. Temesgen et al. (2017) and Alheshibri et al. (2016) propose using the term NB to describe bubbles with a diameter of less than one μm. However, the term “ultrafine bubble” refers to bubbles in the same size range and exhibiting similar properties. Early evidence of the presence of bubbles less than one μm in diameter was reported in seawater by Johnson and Cooke (1981). Since then, most studies involving NBs have focused on methods of NB generation, detection, and characterization (Temesgen et al., 2017).

Nanobubbles have a large surface area per unit volume due to their small size, with a corresponding concentration as high as one hundred million to ten trillion bubbles per milliliter of liquid (Atkinson et al., 2019). Nanobubbles allow for high gas dissolution rates in liquids due to greater internal pressure in the bubble than their environment and high stagnation in the liquid phase (Ushikubo et al., 2010). Additional studies suggest that NBs exhibit a long residence time in solution owing to a negatively charged surface (zeta potential). This surface charge prohibits the coalescence of bubbles, which eventually rise to the surface and dissipate in the atmosphere (Ushikubo et al., 2010, Takahashi et al., 2007). Nanobubbles have been reported to remain in an aqueous solution for weeks and even months (Azevedo et al., 2016; Duval et al., 2012), but other studies have reported a rapid loss of oxygen in NB-oxygenated water (Langenfeld and Bugbee, 2021; See Chapter 3) attributed to flowing water.

The use of overhead irrigation with NB-oxygenated water to deliver oxygen to the root zone of a sand-based creeping bentgrass (*Agrostis stolonifera* L.) putting green has been investigated in the past with no effect on plant growth (See Chapter 3). More than 50% of the initial DO was lost from the NB-oxygenated irrigation water after passing through an irrigation nozzle and contacting the surface of the putting green. Subsurface irrigation should limit DO loss from NB-oxygenated irrigation water by reducing the amount of turbulent action to which the irrigation water is exposed. Therefore, we tested the hypothesis that subsurface irrigation with NB-oxygenated water sources would increase green turfgrass coverage (GTC), dark green color index (DGCI), root growth, and tissue total-N and nitrate-N content of a sand-based 'Pure Distinction' creeping bentgrass grown in controlled-environment lysimeters.

Materials and Methods

Lysimeter construction and environment

The response of creeping bentgrass to subsurface irrigation with NB-oxygenated water sources was investigated in a controlled greenhouse environment at the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR (36.09° N, 94.17° W) from February through April 2021 (Experimental Run 1) and January through March 2022 (Experimental Run 2). For run 1, the maximum and minimum air temperatures recorded were 40 and 8 °C, respectively, average relative humidity was 58%, and peak photosynthetically active radiation was 1203 to 1290 µmol m^{-2} s^{-1}. For run 2, the maximum and minimum air temperatures recorded were 33 and 8 °C, respectively, average relative humidity was 52%, and peak photosynthetically active radiation was 1143 to 1211 µmol m^{-2} s^{-1}.

Lysimeters were constructed from polyvinyl chloride tubes with an inner diameter of 15 cm and a height of 31.5 cm by capping one end with cheesecloth designed to keep sand from escaping but allow for adequate water movement through the soil profile. A 20-cm layer of sand conforming to United States Golf Association specifications for putting green use (U.S. Golf Association, 2018) was placed in each lysimeter and packed to a uniform bulk density of 1.7 g cm^{-3} (Fig. 4.1). Plugs of mature 'Pure Distinction' creeping bentgrass were extracted to a depth of 10 cm from a previously established sand-based root zone, which also met United States Golf

Association specifications for a sand-based putting green (U.S. Golf Association, 2018), to equal a total column height of 30 cm. Once constructed, lysimeters were set onto 10 cm of gravel inside 27.3-L beverage dispensing containers equipped with spigots for drainage (Fig. 4.2).

Treatment procedure

An NB-injection system (Nano Bubble Technologies (NBT), Sydney, AU) was used to oxygenate both potable water from the Beaver Water District (Lowell, AR) and irrigation pond water from the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR (Appendix A-5). The two main effects of Water (Potable or Pond) and Nanobubbles (NB-oxygenated or untreated) were arranged in a 2 × 2 factorial totaling four treatments that included the following:

Potable water treatments (Potable):

- Oxygenated using the NB injection system (NBT - POT)
- Untreated (POT)

Irrigation-pond water treatments (Pond):

- Oxygenated using the NB injection system (NBT - PND)
- Untreated (PND)

Nanobubble-oxygenated treatments were cycled through the NB injection system delivering 1.5 L min^{-1} of industrial-grade oxygen to a water volume of 94 L, cycling for one hour at a rate of 26 L min^{-1}. Irrigation was conducted via subsurface irrigation by filling each beverage dispenser with its respective water treatment by placing the outlet hose from the NB generator directly into the beverage dispenser (Fig. 4.3). Dissolved oxygen concentrations in the NB-oxygenated treatments ranged from 26-29 mg L^{-1}. Dissolved oxygen concentrations in the untreated water ranged from 7-9 mg L^{-1}. Water was allowed to infiltrate the root zone from the bottom of the lysimeter for a period of 1 hr. The dissolved oxygen (DO) content of irrigation water was measured and recorded after filling beverage dispensers using a portable DO meter (Hannah Instruments, Model HI98193, Woonsocket, RI) and a Clark-type polarographic DO probe with a polytetrafluoroethylene polymer membrane cap (Hannah Instruments, Model HI764073, Woonsocket, RI). The creeping bentgrass was clipped with scissors twice weekly at the surface of the PVC to maintain a 1.5 cm height of cut. Plugs were fertilized weekly with 6.0 kg nitrogen (N) ha^{-1} using complete hydroponic fertilizer 16-4-17 (Oasis Grower Solutions, Kent, OH).

Data Collection

Green turfgrass coverage and dark green color index (DGCI) were evaluated weekly using digital image analysis (DIA) (Karcher & Richardson, 2003; Richardson et al., 2001). Images were obtained using a digital camera (Canon PowerShot G12, Canon Inc., Melville, NY) mounted to a modified light box equipped with a frame (Karcher & Richardson, 2013) by attaching the light box to a purple foam board with a 10 cm diameter cut-out in the center to ensure only turfgrass of interest was being captured in the photo (Figs. 4.4, 4.5). Images collected were then analyzed using TurfAnalyzer (Karcher et al., 2017). For GTC, green pixels were selected based on a hue range of 45 to 125 and a saturation range from 10 to 100. The total number of green pixels was divided by the total number of pixels present in the image to calculate the percent green turfgrass coverage present in the image. Clippings were collected every second week, oven dried for at least 72 hours at 80 °C and weighed. Collected clippings were sent to the University of Arkansas Agricultural Diagnostic Laboratory and analyzed for total-N by combustion (Campbell & Plank, 1992) and nitrate-N using the modified Cataldo method (Cataldo et al., 2008). Eight weeks after trial initiation, root analysis was conducted by removing plugs from lysimeters, cutting roots from verdure, and washing roots of all soil. Root total length, surface area, average diameter, and volume were determined from fresh samples using image scanning analysis (WinRhizo, Regent Instruments, Quebec City, Canada). Roots were then oven dried for at least 72 hr at 80 °C, and total dry weights were recorded.

Experimental design and analysis

Treatments were arranged as a 2 × 2 factorial in a randomized complete block design with four replications of each treatment. Root total length, surface area, average diameter, volume, and dry weight were subjected to analysis of variance (ANOVA) ($P < 0.05$) using PROC MIXED of SAS v. 9.4. Leaf tissue total N and NO_3^-, as well as green turfgrass coverage and DGCI were subjected to repeated measures ANOVA ($P < 0.05$) using PROC GLIMMIX of SAS v. 9.4. For significant effects, treatment means were separated using Fisher's least significant difference test ($\alpha = 0.05$).

Results

Digital image analysis

No significant differences in green turfgrass coverage between treatments were detected in either experimental run (Table 4.1). Green turfgrass coverage ranged from 76 to 99% with a mean of 92%. The DGCI was affected by water source and NB-oxygenation, but the results were inconsistent. In 2021, the Water Source × NB-Oxygenation interaction significantly affected DGCI, as DGCI was greater in the NBT-PND treatment compared to all other treatments (Fig. 4.6), but this was not observed in the second run of the study (Table 4.1).

Root growth

Nanobubble-oxygenation of irrigation water did not affect the measured root growth characteristics of creeping bentgrass. During both experimental runs, root growth was not affected by water source, nanobubble oxygenation or their interaction (Table 4.2, 4.3). In the first experimental run, WinRhizo determined that total root length ranged from 1284 to 2829 cm, with a mean of 2162 cm. Total root surface area ranged from 635 to 691 cm^2 with a mean of 664 cm^2. Root average diameter ranged from 0.8 to 1.6 mm with a mean of 0.9 mm. Total root volume ranged from 12.5 to 26.5 cm^3 with a mean of 16.7 cm^3. Total root weight ranged from 1.2 to 5.0 g with a mean of 2.9 g. In the second experimental run, WinRhizo determined that total root length ranged from 831 to 2397 cm, with a mean of 1381 cm. Total root surface area ranged from 534 to 667 cm^2 with a mean of 598 cm^2. Root average diameter ranged from 0.88 to 2 mm with a mean of 1.4 mm. Total root volume ranged from 14.2 to 34.6 cm^3 with a mean of 22.0 cm^3. Total root weight ranged from 4 to 8.1 g with a mean of 6.25 g.

Leaf tissue total N and nitrate-N content

Nanobubble-oxygenation of irrigation water did not affect clipping N in any season. During both experimental runs, leaf tissue total-N and nitrate-N were only affected by Date (Table 4.4). Total N in leaf tissue ranged from 1.5 to 5.17 % with a mean of 3.8% in the first experimental run and 1.34 to 3.4% with a mean of 2.7% in the second run. Tissue nitrate-N ranged from 325 to 900 mg kg^{-1} with a mean of 593 mg kg^{-1} in the first experimental run and 235 to 507 mg kg^{-1} with a mean of 393 mg kg^{-1} in the second run.

Discussion

In previous research (see Chapter 3), significant DO was lost from NB-oxygenated irrigation water in the time between the generation of the water and the water making contact with the experimental putting green during irrigation events. Dissolved oxygen loss from irrigation water in this research was successfully reduced using subsurface irrigation with water pumped directly from the NB generator to the lysimeters (Tables A2 – A5). It was hypothesized that increasing the dissolved oxygen delivered to the root zone would result in detectable plant health benefits. However, the increased DO (26-29 mg L^{-1}) in the irrigation water in this research compared to the overhead irrigation trial (12-15 mg L^{-1}) (see Chapter 3) did not elicit any positive plant growth responses. No differences in green turfgrass coverage, dark green color index, root growth, or tissue N were attributed to subsurface irrigation with NB-oxygenated irrigation water.

As the air in the soil pores is displaced by water during irrigation or rain events, temporary hypoxia in the root zone can occur (Bhattarai et al., 2005). However, according to Morard and Silvestre (1996), temporary oxygen deficiency (a few hours) does not cause irreversible nutritional stress in plants, but extended periods of hypoxia (several days) provoke a decrease in growth that results in a significant reduction in crop yield. Although soil oxygen content was not monitored directly in this study, symptoms of oxygen deficiency were never observed, and it appears that no oxygen deficiency occurred throughout either trial.

A lack of plant response to oxygenated irrigation water in other crops, including turf, was also observed by Yafuso and Fisher (2017), Bonachella et al. (2010), Ehret et al. (2010), Bonachella et al. (2005), and Guertal (2002), who all observed little, or no plant health benefits from irrigating with oxygenated water in course-textured or porous media. The lack of plant response to NB-oxygenated irrigation water could be attributed to the absence of an oxygen deficiency or hypoxic periods of sufficient length or intensity to elicit a plant growth response from oxygenated irrigation water (Bonachella et al., 2010).

Research that demonstrated the beneficial effects of aerated water on the growth of creeping bentgrass was conducted by Kurtz and Kneebone (1980). The authors cultivated creeping bentgrass grown in water baths of varying aeration levels, and enhanced bentgrass growth was observed in the water containing the greatest DO concentration. Our research fundamentally differs from Kurtz and Kneebone in that our bentgrass had access to oxygen in soil pores and more closely simulated the conditions to which bentgrass would be exposed in a sand-based root zone. Because the creeping bentgrass in our research had access to oxygen sources other than oxygen dissolved in our irrigation water, the aerated water failed to elicit a growth response similar to that observed by Kurtz and Kneebone.

It is important to note that subsurface irrigation with oxygenated or aerated water has benefitted many crops, including increased yield and water use efficiency of potato (*Solanum* tuberosum) in a sandy clay loam (Shahein et al., 2014), increased corn yield in a water-logged vermiculite substrate (Lei et al., 2016), increased yield of cotton (*Gossypium hirsutum*), soybean (*Glycine max*), and zucchini (*Cucurbita pepo*) in saturated, heavy clay soil (Bhattarai et al., 2004), and increased pineapple (*Ananas comosas*) fresh biomass and dry matter weight in a loamy sand soil (Chen et al., 2011). In these studies, soil conditions differed from those in our research. The soil in our research was more than 60% sand and did not remain waterlogged for extended periods, two commonalities observed in research demonstrating increased plant growth from oxygenated irrigation water. A plant growth response to NB-oxygenated irrigation water would be more likely when grown in finer-textured soils than a sand-based putting green.

Conclusions

Our hypothesis was rejected as subsurface irrigation with NB-oxygenated water failed to increase GTC, DGCI, root growth characteristics, and leaf tissue total-N and nitrate-N of creeping bentgrass grown in sand-based lysimeters. The properties of NBs allowed for efficient water oxygenation to nearly 30 mg L^{-1} DO, but NBs did not affect plant growth in these experiments. This study suggests that, although not measured directly, the amount of oxygen present in the course-textured soil of a sand-based putting green root zone was not a limiting factor affecting the growth of creeping bentgrass. Providing additional oxygen through subsurface irrigation to a sand-based root zone constructed to USGA specifications will not result in increased root growth or plant health characteristics of a creeping bentgrass putting green. Further research investigating irrigation with oxygenated water sources should focus on turfgrasses managed on fine-textured soils, which are more likely to experience root zone oxygen deficiencies.

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Table 4.1. Analysis of variance for dark green color index and green turfgrass coverage of lysimeter-grown creeping bentgrass as affected by irrigation water source and nanobubble-oxygenation of irrigation water.

Effect	Dark green color index	% Green turfgrass coverage	Dark green color index	% Green turfgrass coverage
	Exp. 1		Exp. 2	
	------------------------------------- *P* > *F*		-------------------------------------	
Water Source	0.0469	NS	NS	NS
NB-Oxygenation	NS	NS	NS	NS
Water Source × NB-Oxygenation	0.0413	NS	NS	NS
Date	<0.0001	<.0001	<.0001	<.0001
Water Source × Date	NS	NS	NS	NS
NB-Oxygenation × Date	NS	NS	NS	NS
Water Source × NB-Oxygenation × Date	NS	NS	NS	NS

[a] NS, nonsignificant at the 0.05 probability level.

Table 4.2. Analysis of variance for root growth parameters of lysimeter-grown creeping bentgrass as affected by irrigation water source and nanobubble-oxygenation of irrigation water in 2022.

Effect	Weight		Length		Surface area		Avg. diameter		Volume	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
	---------- *P* > *F* ----------									
Water Source	NS[a]	NS	NS	NS	NS	NS	NS	NS	NS	NS
NB-Oxygenation	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Water Source ×NB-Oxygenation	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] NS, Nonsignificant at the 0.05 probability level.

Table 4.3. Analysis of variance for leaf tissue total N and NO_3^- content of lysimeter-grown creeping bentgrass as affected by irrigation water source and nanobubble-oxygenation of irrigation water.

Effect	Leaf tissue total N	Leaf tissue NO_3^-	Leaf tissue total N	Leaf tissue NO_3^-
	Exp. 1		Exp. 2	
	------------------------------------ $P > F$		------------------------------------	
Water Source	NS[a]	NS	NS	NS
NB-Oxygenation	NS	NS	NS	NS
Water Source × NB-Oxygenation	NS	NS	NS	NS
Date	<0.0001	0.012	<0.0001	<0.0001
Water Source × Date	NS	NS	NS	NS
NB-Oxygenation × Date	NS	NS	NS	NS
Water Source × NB-Oxygenation × Date	NS	NS	NS	NS

[a] NS, Nonsignificant at the 0.05 probability level.

Figure 4.1. An example of a PVC lysimeter packed with 20 cm of sand placed on 10 cm of gravel within a beverage dispensing bucket to investigate the effect of subsurface nanobubble-oxygenated irrigation water on creeping bentgrass growth.

Figure 4.2. Beverage dispensers with spigots filled with 10 cm of gravel used for subsurface irrigation of creeping bentgrass grown in sand-based lysimeters with nanobubble-oxygenated irrigation water.

Figure 4.3. Nanobubble-oxygenated irrigation water being applied directly from the nanobubble generator to the subsurface irrigation beverage dispenser (through the red hose, center left) to investigate the effects of nanobubble-oxygenated irrigation water on creeping bentgrass grown in sand-based lysimeters.

Figure 4.4. Modified light box used to capture images digitally analyzed for green turfgrass coverage and the dark green color index of creeping bentgrass grown in sand-based lysimeters.

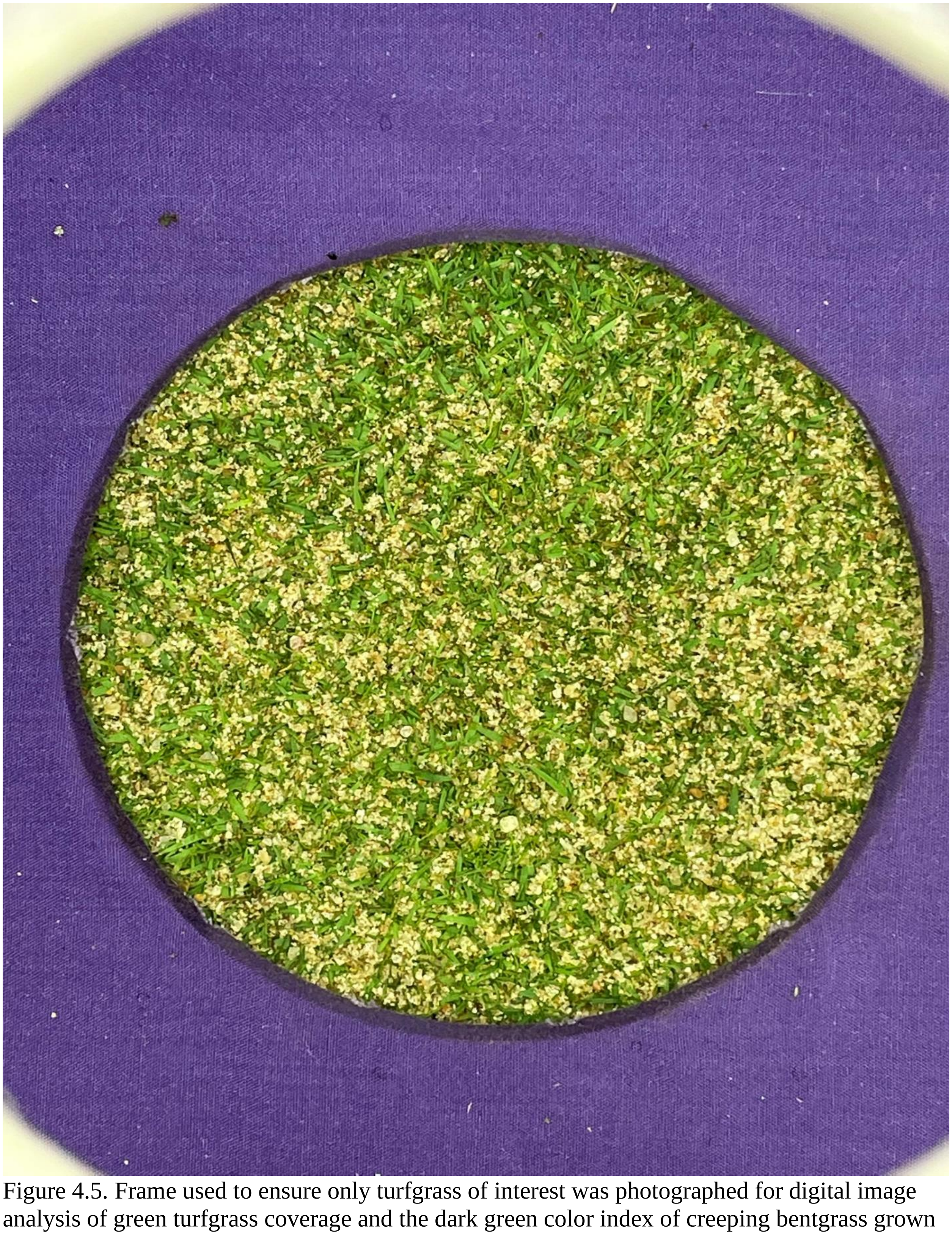

Figure 4.5. Frame used to ensure only turfgrass of interest was photographed for digital image analysis of green turfgrass coverage and the dark green color index of creeping bentgrass grown in sand-based lysimeters.

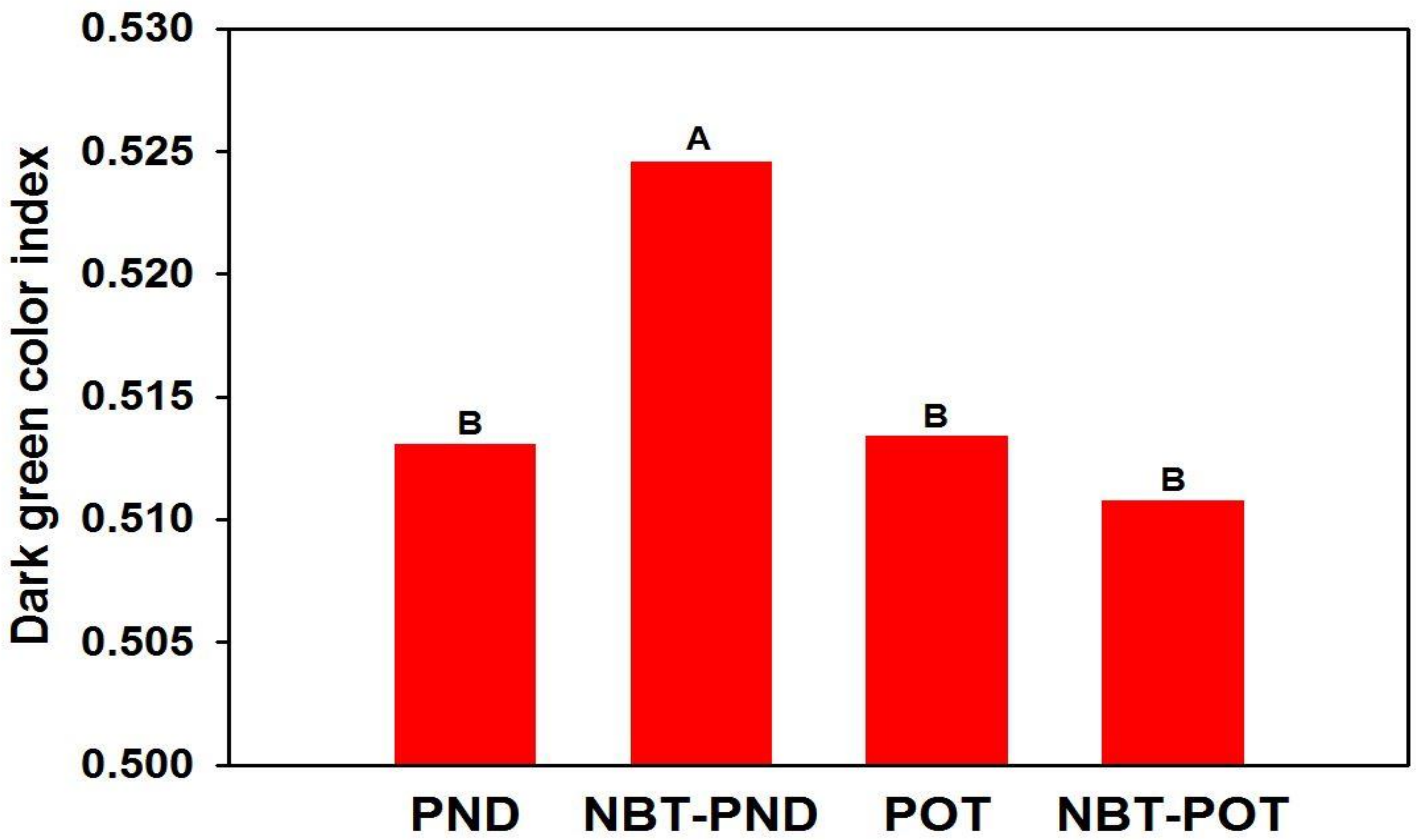

Figure 4.6. Effect of the Water Source [potable (POT) or pond (PND)] × NB-Oxygenation [nanobubble-oxygenated (NBT) or untreated] interaction on the dark green color index of creeping bentgrass grown in a sand-based lysimeter in a controlled environment. Values with the same letter are not significantly different according to LSD ($\alpha = 0.05$).

Chapter 5. Summary

Conclusions and future research

In the current state of technology, NB oxygenation of irrigation water using membrane-based NB injection systems has many limitations that hinder the widespread applicability of NB water oxygenation for golf courses or other horticultural applications. Membrane-based systems can be prone to scale build-up, causing membrane clogging (Ron Pote, NanoOxygen Systems, Personal Communication). Because of the residue build-up within the membrane, membranes require frequent cleaning and inspection. Frequent inspection and cleaning of membranes may result in unacceptable NB-generator downtime for large-scale operations that require frequent oxygenation of large water volumes.

Another limitation of NB oxygenation is the inability to quickly and reliably detect the presence of NBs immediately after treating water. Detection of NBs must overcome two main obstacles to ensure reliable NB detection: 1) Technology for nanoscopic particle detection is expensive, not widely available, and highly technical; 2) An inability to differentiate between nanoscopic particles and NBs during water analysis. The second limitation will be significant for a horticultural or agricultural system, as water quality will have a major effect on the reliability of the analysis.

Nanoparticle detection systems like the NanoSight NS300 (Malvern Panalytical, Salisbury, United Kingdom) also require someone with special training in order to analyze water samples. Additionally, systems like the NS300 can be cost-prohibitive and would become another large investment in addition to installing an NB generator. With no detection tools immediately available, water samples would need to be sent to a lab for analysis to confirm the proper function of an NB generator. Sending samples off for analysis can take multiple days before results are determined.

Not having the capability of differentiating nanoscopic bodies in water sources requires that NB-oxygenated water be compared to a potable-water control, which serves as a baseline from which to compare the NB-treated water. Until these limitations can be overcome, measuring the DO content of the treated water remains the only quickly accessible technique for estimating the efficacy of an NB generator. Even if DO levels are increased in NB-treated water, it does not confirm the presence of NBs in the water.

The properties of NBs, such as a negative surface charge, long stagnation time, and a large interfacial surface area, should theoretically result in longer DO retention compared to a standard aeration method. In our research, two membrane-based NB injection systems failed to increase the retention time of DO in potable water stored at three different temperatures. Dissolved oxygen retention was likely reduced in NB treatments due to the presence of various solutes affecting the properties of the NBs in treated water. Irrigation ponds and hydroponic nutrient solutions contain many solutes that could affect the long-term stability of NBs. Practitioners irrigating crops with non-pure water sources should not expect increased DO retention times compared to standard aeration methods in water aerated with membrane-based NB injection systems.

One limitation of this research was that the water used was not oxygenated at elevated temperatures. Treated water was oxygenated at much colder temperatures than the temperature of the growth chambers in which the water was placed. Because irrigation ponds and hydroponic nutrient solutions are subjected to high-temperature extremes during the summer months, future research should investigate NB injection systems and their ability to oxygenate water at elevated temperatures during the oxygenation process. There may be applications for NB injection systems in the agricultural and horticultural industries if NB injection systems allow for increased gas transfer compared to standard aeration methods at elevated water temperatures.

Infiltration rates of NB-oxygenated water sources were never greater than those of untreated water sources. The experimental putting green on which measurements were conducted was not displaying the characteristic symptoms of hydrophobic soil, which may have resulted in a lack of treatment effect. Future research investigating infiltration rates of NB-oxygenated water in putting greens displaying symptoms of hydrophobic soil could help elicit a treatment response. Even so, membrane-based NB technology in its current state would not be worth the installation investment for the sole purpose of increasing water infiltration in sand-based putting greens due to the high cost (upwards of $300,000) and frequent maintenance of the membrane-based systems.

Our results indicate that NB-oxygenation and the resultant increase in DO in the irrigation water will not result in plant health benefits in sand-based root zones. Most of the significant treatment effects in our work resulted from differences in water sources and were not attributed to water oxygenation. These findings do not differ from the research of other authors, who investigated oxygenated water sources for putting green irrigation, in that plant health benefits from oxygenated irrigation water were either short-lived or nonexistent. The coarse texture of a sand-based putting green root zone likely provided adequate oxygen to the creeping bentgrass roots through the extensive network of macropores present in the root zone.

Nanobubble oxygenation remains a highly efficient means of oxygenating large volumes of water in relatively short periods. Nanobubble-oxygenation may potentially be used in the remediation of irrigation ponds that suffer from the effects of low DO levels during the heat of the summer months. Future research should include investigations into the effect of NB-oxygenation of water for irrigation of golf course turf that is not being grown in a sand-based or sand-capped growing medium and irrigation of other grass species commonly used on golf courses and athletic fields. It would also be beneficial to investigate the fate of the DO that was initially contained in the irrigation water after it reaches the soil surface and begins to infiltrate the soil, as our soil oxygen sensors did not reliably sense increases in soil oxygen after irrigation with NB-oxygenated water.

Appendix

Table A1. Water quality parameters for water used to investigate dissolved oxygen retention time of two nanobubble injection systems and an air stone.

Parameter	Value
Iron (mg L^{-1})	0.01
Manganese (mg L^{-1})	<0.02
pH	8.3
Conductivity (µS cm^{-1})	187
Alkalinity (mg L^{-1} as $CaCO_3$)	32.0
Fluoride (mg L^{-1})	0.68
Chloride (mg L^{-1})	7.26
Sulfate (mg L^{-1})	25.7
Nitrate-N (mg L^{-1})	0.064
Calcium (mg L^{-1} as $CaCO_3$)	32
Total dissolved solids (mg L^{-1})	130

Table A2. Monthly mean water quality parameters for irrigation water used to investigate the effects of nanobubble-oxygenated irrigation on creeping bentgrass growth in 2019.

Parameter	**June**	**July**	**August**	**September**
Iron (mg L^{-1})	<0.01	<0.01	<0.01	<0.01
Manganese (mg L^{-1})	<0.002	<0.002	<0.002	<0.002
pH	8.3	8.6	8.5	8.4
Conductivity (µS cm^{-1})	177	187	194	202
Alkalinity (mg L^{-1} as $CaCO_3$)	40.0	45.0	51.0	55.0
Fluoride (mg L^{-1})	0.65	0.67	0.66	0.67
Chloride (mg L^{-1})	6.82	7.16	7.3	7.4
Sulfate (mg L^{-1})	25.3	26.1	25.1	25.0
Nitrate-N (mg L^{-1})	0.503	0.239	0.102	0.063
Calcium (mg L^{-1} as $CaCO_3$)	54.0	57.0	65.5	69.5
Total dissolved solids (mg L^{-1})	103	108	117	114
Dissolved oxygen (mg L^{-1})	7.31	6.58	5.64	5.73
Dissolved oxygen w/ nanobubbles (mg L^{-1})	13.85	12.83	12.45	12.36

Table A3. Monthly mean water quality parameters for irrigation water used to investigate the effects of nanobubble-oxygenated irrigation on creeping bentgrass growth in 2020.

	June		July		August		September	
Parameter	**Pond**	**Potable**	**Pond**	**Potable**	**Pond**	**Potable**	**Pond**	**Potable**
Iron	8.78	<0.01	0.32	<0.01	1.36	<0.01	0.07	<0.01
Manganese	5.98	<0.002	1.90	<0.002	1.23	<0.002	0.10	<0.002
pH	6.8	7.5	6.8	8.0	7.1	7.9	6.8	7.6
Conductivity (µS cm^{-1})	222	188	135	194	110	197	93.0	219
SAR (calc.)	0.10	0.36	0.12	0.36	0.22	0.36	0.15	0.36
Alkalinity (as $CaCO_3$)	108	52.0	58.0	52.0	48.0	46.0	34.0	62.0
Bicarbonate (mg L^{-1} as $CaCO_3$)	108	51.8	58.0	51.6	47.9	45.7	34.0	61.8
Fluoride (mg L^{-1})	0.33	0.68	0.28	0.77	0.21	0.63	0.27	0.86
Chloride (mg L^{-1})	2.01	6.77	2.09	6.88	1.79	7.19	2.30	7.82
Sulfate (mg L^{-1})	0.17	25.1	0.37	24.2	0.50	26.7	3.48	25.8
Nitrate-N (mg L^{-1})	0.00	0.38	0.00	0.17	0.01	0.02	0.01	0.03
Magnesium (mg L^{-1})	1.62	1.83	0.89	1.83	0.87	1.76	0.62	1.84
Calcium (mg L^{-1})	27.7	27.6	17.7	28.8	15.0	30.0	14.8	34.2
Sodium (mg L^{-1})	1.93	7.14	1.84	7.33	3.27	7.50	2.14	7.91
Total suspended solids (mg L^{-1})	74.0	6.00	12.0	0.00	61.0	0.00	32.0	0.10
DO[a] (mg L^{-1})	5.20	7.50	4.60	6.50	3.90	6.30	3.80	6.00
DO w/ nanobubbles (mg L^{-1})	13.4	14.1	13	14.1	13.3	13.9	12.7	13.4

[a] Dissolved oxygen content.

Table A4. Monthly mean water quality parameters for irrigation water used to investigate the effects of nanobubble-oxygenated irrigation on creeping bentgrass growth in 2021.

	June		July		August		September	
Parameter	**Pond**	**Potable**	**Pond**	**Potable**	**Pond**	**Potable**	**Pond**	**Potable**
Iron	9.54	<0.01	9.08	<0.01	0.89	<0.01	0.45	<0.01
Manganese	2.21	0.00	2.44	0.00	6.1	0.00	0.27	0.00
pH	6.1	8.0	6.1	8.2	6.6	7.7	7.2	7.7
Conductivity (µS cm^{-1})	148	180	218	197	292	219	149	218
SAR (calc.)	0.24	0.38	0.16	0.37	0.10	0.36	0.15	0.36
Alkalinity (as $CaCO_3$)	58.0	40.0	78.0	50.0	124	56.0	54.0	60.0
Bicarbonate (mg L^{-1} as $CaCO_3$)	58.0	39.0	78.0	49.2	124	55.8	53.9	59.7
Fluoride (mg L^{-1})	0.33	0.80	0.37	0.75	0.30	0.74	0.39	0.79
Chloride (mg L^{-1})	2.37	6.14	3.41	6.91	2.65	7.25	3.09	7.35
Sulfate (mg L^{-1})	0.60	25.3	1.22	26.8	1.54	27.5	4.19	27.5
Nitrate-N (mg L^{-1})	0.01	0.33	0.01	0.13	0.01	0.02	<0.01	0.02
Magnesium (mg L^{-1})	1.05	1.79	1.17	1.87	1.09	1.96	0.70	2.06
Calcium (mg L^{-1})	15.6	24.3	15.3	27.2	38.0	28.0	20.3	31.2
Sodium (mg L^{-1})	3.32	7.21	2.44	7.34	2.37	7.41	2.46	7.70
Total suspended solids (mg L^{-1})	40.0	3.00	88.0	0.00	32.0	0.00	8.0	0.00
DO[a] (mg L^{-1})	4.73	7.60	3.90	6.7	3.60	5.70	3.70	5.80
DO w/ nanobubbles (mg L^{-1})	13.2	14.1	12.5	12.8	12.4	13.3	12.7	12.9

[a] Dissolved oxygen content.

Table A5. Water quality parameters for subsurface irrigation water used for irrigating creeping bentgrass grown in lysimeters constructed with sand-based root zones in 2021 and 2022.

	2021		2022	
Parameter	**Pond**	**Potable**	**Pond**	**Potable**
Iron	0.14	<0.01	0.10	<0.01
Manganese	0.39	<0.01	0.44	<0.01
pH	6.8	7.7	6.9	7.6
Conductivity (µS cm^{-1})	100	215	89	201
SAR (calc.)	0.15	0.41	0.17	0.53
Alkalinity (as $CaCO_3$)	36.0	60.0	39.0	62.9
Bicarbonate (mg L^{-1} as $CaCO_3$)	36.0	59.7	39.0	62.2
Fluoride (mg L^{-1})	0.21	0.72	0.45	0.81
Chloride (mg L^{-1})	2.53	8.65	2.41	7.72
Sulfate (mg L^{-1})	5.05	26.7	4.09	25.1
Nitrate-N (mg L^{-1})	0.11	0.61	0.13	0.66
Magnesium (mg L^{-1})	0.83	2.28	0.92	3.01
Calcium (mg L^{-1})	14.3	31.8	13.9	34.9
Sodium (mg L^{-1})	2.10	8.89	2.35	9.09
Total suspended solids (mg L^{-1})	2.7	<1.0	3.1	<1.0
DO[a] (mg L^{-1})	7.4	8.5	7.0	8.1
DO w/ nanobubbles (mg L^{-1})	27.9	28.2	28.5	29.0

[a] Dissolved oxygen content.