Irrigation of Sand-based Creeping Bentgrass Putting Greens with Nanobubble-oxygenated Water

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KEYWORDS. *Agrostis stolonifera*, digital image analysis, lysimeter, root growth, soil oxygen, subsurface irrigation

ABSTRACT. **Cultural and environmental factors can place creeping bentgrass (*Agrostis stolonifera*) under extreme stress during the summer months. This stress, coupled with the growth adaptation of creeping bentgrass, can result in shallow, poorly rooted stands of turf. To enhance root zone oxygen and rooting of creeping bentgrass, golf courses use methods such as core and solid-tine aerification, and sand topdressing. An additional method of delivering oxygen to the soil could be irrigation with nanobubble-oxygenated water. The properties of nanobubbles (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 more resilient playing surface. The objectives of this study include comparing the effects of irrigation with NB-oxygenated water sources with untreated water sources on creeping bentgrass putting green root zone and plant health characteristics using field and controlled environment experiments. Treatments included NB-oxygenated potable water and irrigation pond water, and untreated potable and irrigation pond water. In the field, NB-oxygenated water did not enhance plant health characteristics of creeping bentgrass. In 1 year, NB-oxygenated water increased the daily mean partial pressure of soil oxygen from 17.48 kPa to 18.21 kPa but soil oxygen was unaffected in the other 2 years of the trial. Subsurface irrigation with NB-oxygenated water did not affect measured plant health characteristics in the greenhouse. NB-oxygenation of irrigation water remains an excellent means of efficiently oxygenating large volumes of water. However, plant health benefits from NB-oxygenated irrigation water were not observed in this research.**

Creeping bentgrass (*Agrostis stolonifera*) is a popular and widely used turfgrass for golf course putting green surfaces because of 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 because of 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. Cultural practices and environmental factors can place creeping bentgrass under extreme stress during the summer period, resulting in shallow, poorly rooted stands of turf (Carrow 1996; Fry and 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 and Schlesinger 1992).

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. Nonetheless, scientists have shown increased yields of 10% to 20% when crops were irrigated with oxygenated water (Du et al. 2018). 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 nonaerated 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. 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 nonturf system, irrigating calibrachoa (*Calibrachoa* ×*hybrida* ‘Aloha Kona Dark Red’) and lobelia (*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 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 100 million to 10 trillion bubbles per milliliter of liquid (Atkinson et al. 2019). NBs

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Table 1. Mean water quality parameters of water used for creeping bentgrass irrigation in the field study from 2019 to 2021 as determined by the Arkansas Water Resources Center Water Quality Laboratory.

		2020		2021	
Parameter	2019	Potable	Pond	Potable	Pond
Iron (mg·L^{-1})[i]	0.002	0	2.63	0	4.99
Manganese (mg·L^{-1})	0.007	0	2.30	0	2.755
pH	8.45	7.75	6.875	7.9	6.5
Conductivity (μS·cm^{-1})	189.8	199.4	139.7	203.4	201.5
Alkalinity (mg·L^{-1} as $CaCO_3$)	47.65	53.0	62.0	51.5	78.5
Fluoride (mg·L^{-1})	0.6647	0.735	0.272	0.77	0.347
Chloride (mg·L^{-1})	7.17	7.165	2.047	6.912	2.88
Sulfate (mg·L^{-1})	25.37	25.45	1.13	26.77	1.885
Nitrate-N (mg·L^{-1})	0.2267	0.15	0.005	0.125	0.008
Calcium (mg·L^{-1} as $CaCO_3$)	61.5	30.15	18.8	27.67	22.3
DO[ii] (mg·L^{-1})	6.3	6.6	4.4	6.5	4.0
DO w/ nanobubbles (mg·L^{-1})	12.87	13.88	13.1	13.27	12.7

[i] 1 mg·L^{-1} = 1 ppm

[ii] Dissolved oxygen concentration. DO levels were monitored at every irrigation event using a portable DO meter.

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 that coalesce and rise to the surface (Takahashi et al. 2007; Ushikubo et al. 2010). NBs have been reported to remain in an aqueous solution for weeks and even months (Azevedo et al. 2016; Duval et al. 2012).

Although NB-oxygenation of irrigation water shows potential for increasing oxygen delivery to plant root systems (Ebina et al. 2013; Liu et al. 2016a, 2016b, 2017; Wang et al. 2021; Wu et al. 2019), little is known about the effects of NB-oxygenated irrigation water on the soil oxygen content and plant growth characteristics in turfgrass systems such as a sand-based putting green. Although anecdotal evidence from golf course superintendents claiming increased putting green quality from the use of NB-oxygenated water has been discussed, there have been no studies that have specifically tested the benefits of NB-oxygenated water. We hypothesized that compared with untreated water, long-term irrigation with NB-oxygenated water would increase the root zone oxygen concentration and increase root and shoot growth of an experimental sand-based creeping bentgrass putting green in field and controlled environment experiments.

Fig. 1. An example of a polyvinyl chloride lysimeter packed with 20 cm (7.87 inches) of sand placed on 10 cm (3.94 inches) of gravel within a beverage-dispensing bucket to investigate the effect of subsurface nanobubble-oxygenated irrigation water on creeping bentgrass growth.

Materials and methods

Field experiment. A 3-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 2019–21. Research was conducted on a mature, sand-based (U.S. Golf Association 2004) creeping bentgrass (cv. Pure Distinction) putting green. Mowing was performed 6 days per week 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, USA). From May through October, nitrogen (N) was applied every second week at a rate of 13 kg·ha^{-1}, alternating between water-soluble urea (Thrive 46N–0P–0K, Mears Fertilizer Inc., El Dorado, KS, USA) and Contec DG granular fertilizer (18N–3.9P–15K, The Andersons, Maumee, OH, USA). 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 2-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.

In 2019, a proprietary membrane-based NB injection system (Nano Bubble Technologies, Sydney, NSW, Australia) was used to oxygenate potable water from the Beaver Water District (Lowell, AR) (Table 1). Nanobubble-oxygenated water was cycled through the NB injection system delivering 1.5 L·min^{-1} of industrial-grade oxygen

Fig. 2. Beverage dispensers with spigots filled with 10 cm (3.94 inches) of gravel used for subsurface irrigation of creeping bentgrass grown in sand-based lysimeters with nanobubble-oxygenated irrigation water.

to a water volume of 100 gal, cycling for a period of 2 hours at a rate of 7 gallons per minute. In 2019, irrigation of creeping bentgrass with NB-oxygenated water was compared with potable tap water in a randomized complete block design with four replications.

In 2020 and 2021, a second water source was added to the study. The membrane-based NB injection system was used to oxygenate both potable water from the Beaver Water District and irrigation pond water from the Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR (Table 1). The two main effects of water source (Potable or Pond) and NB-oxygenation (NB-oxygenated or Untreated) were replicated four times in a randomized complete block design as a 2 × 2 factorial totaling four treatments that included two potable water treatments (Potable) consisting of water oxygenated using the NB injection system (NBT-POT) and untreated water (POT), and two pond water treatments (Pond) of water oxygenated using the NB injection system (NBT-PND) and untreated water (PND).

After treatment, each water treatment was transferred to a 25-gal, 12-V sprayer system (NorthStar ATV 12V Spot Sprayer, Clayton Engineering, Brendale, QLD, Australia) for transport and final delivery of the water to the putting green from a drenching nozzle (Cool Shot Plus, Weathermatic, Garland, TX, USA) delivering 5 gallons per minute. The dissolved oxygen (DO) concentration of the irrigation water used in this research was monitored and recorded at multiple stages of the oxygenation and irrigation process, including in the NB-oxygenation circulation tank, in the irrigation application sprayer tank, and at the surface of the turf after passing through the sprayer pump and hose nozzle, by placing a catch can on the putting green surface and collecting water exiting the hose. DO was measured using a portable DO meter (Model HI98193; Hannah Instruments, Woonsocket, RI, USA) and a Clark-type polarographic DO probe with a polytetrafluoroethylene polymer membrane cap (Model HI764073; Hannah Instruments). Monthly water quality testing was conducted by the Arkansas Water Resources Center Water Quality Laboratory (Fayetteville, AR, USA) for potable and irrigation pond water.

In 2019, irrigation treatments were applied three times per week to replace 140% reference evapotranspiration (ET_o) for the 2-day period before irrigation events to deliver maximum DO to the root zone. Reference evapotranspiration and precipitation were determined using an on-site weather station (WS-2902; Ambient Weather, Chandler, AZ, USA), wherein climatological data were recorded to determine ET_o estimates using the FAO-56 Penman-Monteith equation (Allen et al. 1998). Because of a lack of treatment effects in 2019 and to simulate a more practical irrigation strategy, total ET_o replacement was adjusted in 2020 and 2021, with treatments applied three times per week to replace 100% ET_o for the 2-day period before irrigation events. Irrigation was omitted when precipitation totals were greater than replacement ET_o. Individual plots measured 4 × 4 ft with 1.5-ft alleys to minimize lateral movement of varying water sources in the soil profile.

Greenhouse experiment. The response of creeping bentgrass to subsurface irrigation with NB-oxygenated water sources was also 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 Feb through Apr 2021 (Experimental Run 1) and Jan through Mar 2022 (Experimental Run 2). For Run 1, the maximum and minimum air temperatures recorded were 104 and 46 °F, respectively, average relative humidity was 58%, and peak photosynthetically active radiation (PAR) was

Fig. 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.

Fig. 4. Modified lightbox used to capture images digitally analyzed for green turfgrass coverage and the dark green color index of creeping bentgrass grown in sand-based lysimeters.

1203 to 1290 μmol·m^{-2}·s^{-1}. For Run 2, the maximum and minimum air temperatures recorded were 91 and 46 °F, respectively, average relative humidity was 52%, and peak PAR was 1143 to 1211 μmol·m^{-2}·s^{-1}. No supplemental lighting was provided during either experimental run.

Lysimeters were constructed from polyvinyl chloride (PVC) tubes with an inner diameter of 6 inches and a height of 12 inches by capping one end with cheesecloth designed to keep sand from escaping but allow for adequate water movement through the soil profile. An 8-inch layer of sand conforming to U.S. 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. 1). Plugs of mature 'Pure Distinction' creeping bentgrass were extracted to a depth of 4 inches from a previously established sand-based root zone, which also met U.S. Golf Association specifications for a sand-based putting green (U.S. Golf Association 2018), to equal a total column height of 12 inches. Once constructed, lysimeters were set onto a 4-inch bed of gravel inside 7-gallon beverage-dispensing containers equipped with spigots for drainage (Fig. 2).

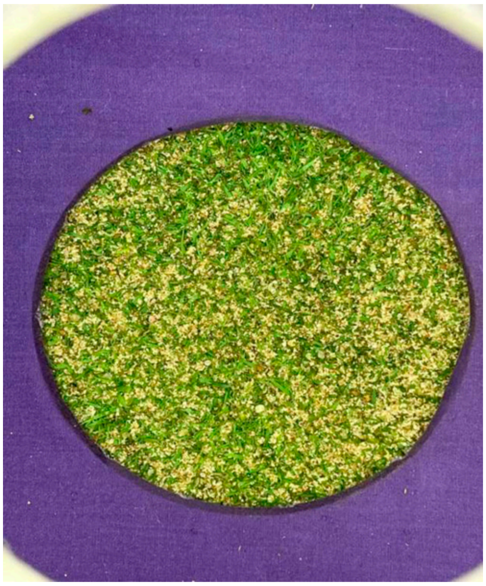

Fig. 5. Frame used to ensure only turfgrass of interest was photographed for digital image analysis of green turfgrass coverage and the dark green color index of creeping bentgrass grown in sand-based lysimeters.

The two main effects of water source (Potable or Pond) and NB-oxygenation (NB-oxygenated or untreated) were replicated four times in a randomized complete block design as a 2 × 2 factorial with the same treatment structure as the field trial. NB-oxygenated treatments were cycled through the NB injection system delivering 1.5 L·min^{-1} of industrial-grade oxygen to a water volume of 25 gallons, cycling for 1 hour at a rate of 7 gallons per minute. 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. 3). Water was allowed to infiltrate the root zone from the bottom of the lysimeter for a period of 1 hour. The dissolved oxygen concentration of irrigation water was measured and recorded after filling beverage dispensers using a portable DO meter (Model HI98193; Hannah Instruments) and a Clark-type polarographic DO probe with a polytetrafluoroethylene polymer membrane cap (Model HI764073; Hannah Instruments). The creeping bentgrass was clipped with scissors twice weekly at the surface of the PVC to maintain a 0.5-inch height of cut. Plugs were fertilized weekly at 0.1 lb/1000 ft^2 nitrogen using complete hydroponic fertilizer 16N–1.7P–14K (Oasis Grower Solutions, Kent, OH, USA).

Data collection. In the field, soil oxygen levels, reported as the partial pressure of oxygen (PPSO), were continuously monitored in two of the four replications at a 6-inch soil depth in 2019 and 2020 and a 3-inch soil depth in 2021, using eight permanently installed oxygen sensors (Apogee SO-110 Soil Response Thermistor Reference Oxygen Sensors; Apogee Instruments, Logan, UT, USA) connected to a data logger and multiplexer (CR1000 and AM16/32b; Campbell Scientific, Logan, UT, USA). Daily average PPSO was determined and used for statistical

Table 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 partial pressure of soil oxygen in a sand-based creeping bentgrass putting green irrigated with nanobubble-oxygenated water during the summers of 2019–21.

	Soil oxygen		
Effect	**2019**	**2020**	**2021**
		$P > F$	
Water Source	NA[i]	NS	NS
NB-Oxygenation	*	*	NS
Water Source × NB-Oxygenation	NA	NS	NS
Date	***	***	***
Water Source × Date	NA	NS	NS
NB-Oxygenation × Date	NS[ii]	NS	NS
Water Source × NB-Oxy × Date	NA	NS	NS

[i] NA, not applicable.

[ii] NS, *, *** nonsignificant or significant at $P \leq 0.05$, 0.001, respectively.

comparison. Green turfgrass coverage (GTC) and dark green color index (DGCI) were evaluated weekly using digital image analysis (DIA) (Karcher and Richardson 2003; Richardson et al. 2001). Images were obtained using a digital camera (Canon PowerShot G12; Canon Inc., Melville, NY, USA) 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 GTC 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 h at 175 °F and weighed. Before 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 and Plank 1992) and nitrate-N using the modified Cataldo method (Cataldo et al. 2008). Root samples that measured 7 inches in depth, 3 inches in width, and 0.5 inches in thickness were collected monthly using a soil profiler (MPS1-S; Turf-Tec International, Tallahassee, FL, USA) 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, QC, Canada).

In the greenhouse, GTC and DGCI were evaluated weekly using DIA (Karcher and Richardson 2003; Richardson et al. 2001). Images were obtained using a digital camera (Canon PowerShot G12) mounted to a modified lightbox equipped with a frame (Karcher and Richardson 2013) by attaching the lightbox to a purple foam board with a 4-inch diameter cutout in the center to ensure only turfgrass of interest was being captured in the photo (Figs. 4 and 5). Images were subjected to the same analysis for DGCI and GTC as the field trial. Clippings were collected every second week, oven-dried for at least 72 h at 175 °F, and weighed. Collected clippings were sent to the University of Arkansas Agricultural Diagnostic Laboratory and analyzed for total-N by combustion (Campbell and 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). Roots were then oven-dried for at least 72 h at 175 °F, and total dry weights were recorded.

Table 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 the dark green color index (DGCI) and green turfgrass coverage (GTC) of a sand-based creeping bentgrass putting green during the summers of 2019–21.

	DGCI	GTC	GCI	GTC	GCI	GTC
Effect	**2019**		**2020**		**2021**	
			$P > F$			
Water Source	NA[i]	NA	NS	NS	0.0226	NS
NB-Oxygenation	NS[ii]	NS	*	NS	NS	NS
Water Source × NB-Oxygenation	NA	NA	NS	NS	NS	NS
Date	***	NS	***	***	***	***
Water Source × Date	NA	NA	*	NS	*	NS
NB-Oxygenation × Date	NS	NS	NS	NS	NS	NS
Water Source × NB-Oxy × Date	NA	NA	NS	NS	***	NS

[i] NA, not applicable.

[ii] NS, *, *** nonsignificant or significant at $P \leq 0.05$, 0.001, respectively.

DATA ANALYSIS. Field data were analyzed separately each year due to inconsistencies in evaluation dates and methodologies between years. To best estimate cumulative treatment effects throughout the summer, the effects of water source, NB-oxygenation, and their interaction on soil oxygen content, GTC, DGCI, root morphology, clipping yield, and clipping N content were analyzed using repeated measures analysis of variance and the general linear mixed model procedure (PROC GLIMMIX) ($P < 0.05$) using a statistical program (SAS version 9.4; SAS Institute Inc., Cary, NC, USA) with date included in the analysis. Where appropriate, treatment means were separated using Fisher's least significant difference test ($\alpha = 0.05$).

Data collected in the greenhouse for 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 nitrate, as well as GTC and DGCI were subjected to repeated measures ANOVA ($P < 0.05$) using PROC GLIMMIX of SAS version 9.4. For

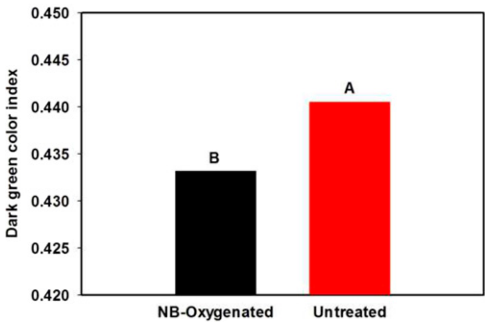

Fig. 6. Effect of nanobubble (NB)-oxygenation of irrigation water sources on the dark green color index of a sand-based creeping bentgrass putting green during the summer of 2020 in Fayetteville, AR. Different letters indicate a significant difference between treatment means ($P < 0.05$).

significant effects, treatment means were separated using Fisher's least significant difference test (α = 0.05).

Results

FIELD EXPERIMENT. Dissolved oxygen concentrations in the NB-oxygenated treatments ranged from 12 to 14 mg·L^{-1}. Dissolved oxygen concentrations in untreated water sources ranged from 4 to 7 mg·L^{-1} (Table 1). NB-oxygenated irrigation water inconsistently affected the PPSO over three seasons (Table 2). 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 with untreated water sources (20.3 kPa). In 2021, no effect or interaction significantly affected PPSO.

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). DGCI was inconsistently affected by NB-oxygenation of irrigation water over three seasons of research (Table 3). In 2019, DGCI was only affected by Date (Table 3). In 2020, the main effect of NB-oxygenation reduced the DGCI of NB-oxygenated water sources (0.4332) compared with untreated sources (0.4405) (Fig. 6). The Water Source × Date interaction in 2020 resulted in a significant increase in DGCI for Potable treatments (0.6724) compared with Pond treatments (0.6501) on one sampling date (Fig. 7). In 2021, DGCI was affected by the highest order interaction of Water Source × NB-oxygenation × Date (Table 3). This interaction resulted in a significantly greater DGCI in the PND treatment compared with other treatments on multiple dates (Fig. 8).

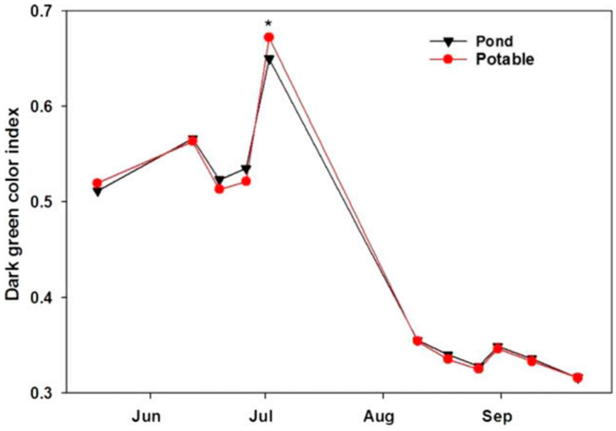

Fig. 7. Effect of the Water Source × Date interaction on the dark green color index of a sand-based creeping bentgrass putting green during the summer of 2020 in Fayetteville, AR. Asterisk indicates date with a significant difference between treatment means ($P < 0.05$).

NB-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 4). Clipping total-N was only affected by the Water Source × Date interaction in 2021 (Table 4). The interaction of Water Source × Date resulted in greater clipping total-N in Potable water treatments compared with Pond water treatments on one date in 2021 (Fig. 9). Clipping nitrate-N was unaffected by treatment other than Date in both seasons (Table 4).

NB-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 (Table 5).

GREENHOUSE EXPERIMENT. Dissolved oxygen concentrations in the NB-oxygenated treatments ranged from 26 to 29 mg·L^{-1}. Dissolved oxygen concentrations in the untreated water ranged from 7 to 9 mg·L^{-1}. No significant differences in GTC between treatments were detected in either experimental run (Table 6). The DGCI was affected by water source and NB-oxygenation, but the results were inconsistent. In 2021, the Water Source × NB-oxygenation interaction resulted in a significant increase of the DGCI for NBT-PND treatment compared with all other treatments (Fig. 10), but this was not observed in the second run of the study (Table 6).

NB-oxygenation of irrigation water did not affect the measured root growth characteristics of creeping bentgrass. During both experimental runs, root growth was unaffected by Water Source, NB-oxygenation, or their interaction (data not shown).

NB-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 7). Total-N in leaf tissue ranged from 1.5% to 5.17% in the first experimental run and 1.34% to 3.4% in the second run. Tissue nitrate-N ranged from 325 to 900 mg·kg^{-1} in the first experimental run and 235 to 507 in the second run.

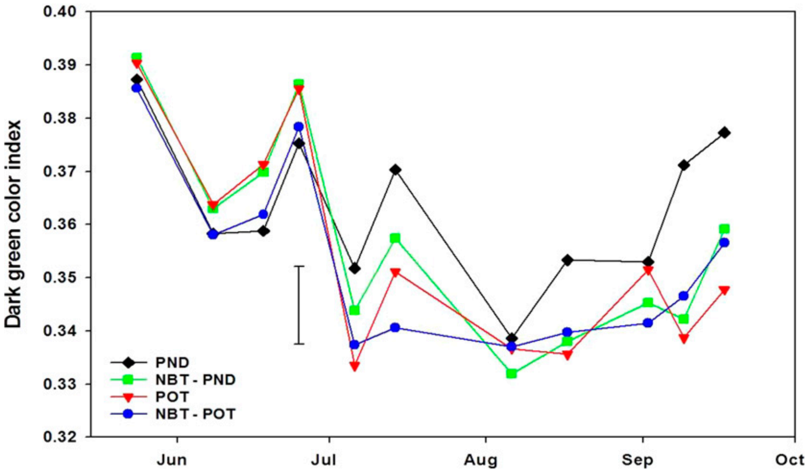

Fig. 8. Effect of the Water Source [potable (POT) or pond (PND)] × NB-Oxygenation [nanobubble oxygenated (NBT) or untreated] × Date interaction on the dark green color index of a sand-based creeping bentgrass putting green during the summer of 2021 in Fayetteville, AR. Error bar represents the least significant difference for comparing means (α = 0.05).

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 and 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 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 1 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 (2002) 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 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, an average of 55% of the initial DO concentration was lost from the NB-oxygenated water when it finally reached the turf surface. Even so, the DO content of NB-oxygenated irrigation water caught on the putting green surface consistently measured more than double the level of DO in untreated water (Table 1). Therefore, it is unclear why the increased DO in the irrigation water was not detected using the soil oxygen response thermistors.

The lack of consistently detectable differences in PPSO suggests that the oxygen contained in the NB-oxygenated treatments was subject to one or a combination of fates, including 1) being consumed immediately by soil organisms and redox processes; 2) remaining in the root zone above a 3-inch depth; 3) gassing off into the atmosphere during infiltration; or 4) not detectable by the soil oxygen sensors. Whether DO was delivered in sufficient quantity to 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 GTC of our experimental plots was never observed to be below 95% on any sampling date in all 3 years of research. If DO was increased in our experimental putting green, the lack of plant response to NB-oxygenated irrigation suggests that a sufficient oxygen supply was present in the soil due to the large number of air-filled pores, typical of sand-based putting green root zones. Several previous studies conducted in course-textured or porous media also observed little or no plant health benefits from irrigating with oxygenated water (Bonachela et al. 2005, 2010; Ehret et al. 2010; Guertal 2002; Yafuso and Fisher 2017).

The greater DGCI values observed in the PND treatment in 2021 can be attributed to a greening effect caused by

Table 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 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.

	Clipping dry wt			Total nitrogen		Nitrate content	
Effect	2019	2020	2021	2020	2021	2020	2021
				$P > F$			
Water Source	NA[i]	NS	NS	NS	NS	NS	*
NB-Oxygenation	NS[ii]	NS	NS	NS	NS	NS	NS
Water Source × NB-Oxygenation	NA	NS	NS	NS	NS	NS	NS
Date	***	***	***	***	***	***	***
Water Source × Date	NA	NS	NS	NS	*	NS	NS
NB-Oxygenation × Date	NS	NS	NS	NS	NS	NS	NS
Water Source × NB-Oxygenation × Date	NA	NS	NS	NS	NS	NS	NS

[i] NA, not applicable.

[ii] NS, *, *** nonsignificant or significant at $P \leq 0.05$, 0.001, respectively.

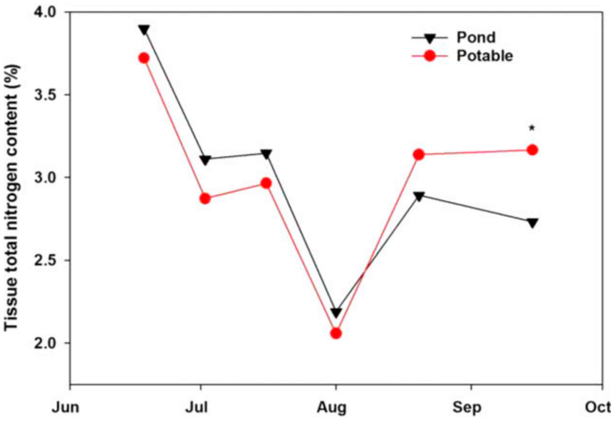

Fig. 9. Effect of Water Source (Potable or Pond) × Date on the leaf tissue total nitrogen content of a sand-based creeping bentgrass putting green during the summer of 2021 in Fayetteville, AR. Asterisk indicates date with a significant difference between treatment means ($P < 0.05$).

the numerically greater iron content in the pond water source compared with the potable water source (Table 1). 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.

In the greenhouse experiments, DO loss from irrigation water was successfully reduced using subsurface irrigation with water pumped directly from the NB generator to the lysimeters. Based on the oxygen losses observed during irrigation in the field studies, it was hypothesized that increasing the DO delivered to the root zone would result in detectable plant health benefits; however, the increased DO (26–29 $\text{mg}\cdot\text{L}^{-1}$) in the irrigation water in this research compared with the overhead irrigation trial (12–14 $\text{mg}\cdot\text{L}^{-1}$) also did not elicit any positive plant growth responses. No differences in GTC, DGCI, 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 the greenhouse study, symptoms of oxygen deficiency were never observed, and it appears that no oxygen deficiency occurred throughout either trial. 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 (Bonachela 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 creeping bentgrass growth was observed in the water containing the greatest DO concentration. Our research fundamentally differs from Kurtz and Kneebone (1980) in that our creeping bentgrass had access to oxygen in soil pores and more closely simulated the conditions to which creeping 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 like that observed by Kurtz and Kneebone (1980).

It is important to note that subsurface irrigation with oxygenated or aerated water has benefited many crops, including increased yield and water use efficiency of potato (*Solanum tuberosum*) in a sandy clay loam (Abuarab et al. 2014); increased corn (*Zea mays*) yield in a waterlogged 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 were finer-textured and would

Table 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 2019–21.

	Length			Volume			Mass	
Effect	2019	2020	2021	2019	2020	2021	2020	2021
				$P > F$				
Water Source	NA[i]	NS	NS	NA	NS	NS	NS	NS
NB-Oxygenation	NS[ii]	NS	NS	NS	NS	NS	NS	NS
Water Source × NB-Oxygenation	NA	NS	NS	NA	NS	NS	NS	NS
Date	NS	***	***	***	***	***	***	***
Water Source × Date	NA	NS	NS	NA	NS	NS	NS	NS
NB-Oxygenation × Date	NS	NS	NS	NS	NS	NS	NS	NS
Water Source × NB-Oxygenation × Date	NA	NS	NS	NA	NS	NS	NS	NS

[i] NA, not applicable.

[ii] NS, *** nonsignificant or significant at $P \leq 0.001$, respectively.

Table 6. **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.**

	Dark green color index	% Green turfgrass coverage	Dark green color index	% Green turfgrass coverage
Effect	**Run 1**		**Run 2**	
		$P > F$		
Water Source	*[i]	NS	NS	NS
NB-Oxygenation	NS	NS	NS	NS
Water Source × NB-Oxygenation	*	NS	NS	NS
Date	***	***	***	***
Water Source × Date	NS	NS	NS	NS
NB-Oxygenation × Date	NS	NS	NS	NS
Water Source × NB-Oxy × Date	NS	NS	NS	NS

[i] NS, *, *** nonsignificant or significant at $P \leq 0.05$, 0.001, respectively.

Table 7. **Analysis of variance for leaf tissue total nitrogen (N) and nitrate nitrogen (NO_3^-) content of lysimeter-grown creeping bentgrass as affected by irrigation water source and nanobubble-oxygenation of irrigation water.**

	Leaf tissue total N	Leaf tissue NO_3^-	Leaf tissue total N	Leaf tissue NO_3^-
Effect	**Exp. 1**		**Exp. 2**	
		$P > F$		
Water Source	NS[i]	NS	NS	NS
NB-Oxygenation	NS	NS	NS	NS
Water Source × NB-Oxygenation	NS	NS	NS	NS
Date	***	*	***	***
Water Source × Date	NS	NS	NS	NS
NB-Oxygenation × Date	NS	NS	NS	NS
Water Source × NB-Oxy × Date	NS	NS	NS	NS

[i] NS, *, *** nonsignificant or significant at $P \leq 0.05$, 0.001, respectively.

be characterized by an increase in small, water-filled pores and a decrease in air-filled pores. The soil in our research was more than 90% sand and did not remain waterlogged for extended periods, which may help explain the lack of plant response to NB-oxygenated irrigation water.

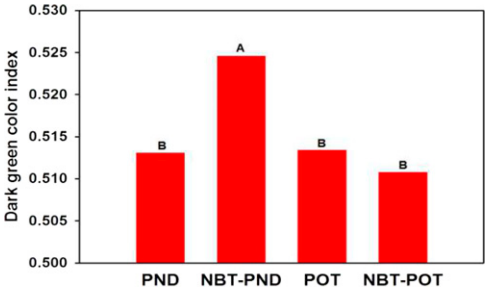

Fig. 10. Effect of the Water Source [potable (POT) or pond (PND)] × NB-Oxygenation [nanobubble-oxygenated (NBT) or untreated] interaction on the dark green color index of creeping bentgrass grown in a sand-based lysimeter in a controlled environment. Values with the same letter are not significantly different according to LSD (α = 0.05).

Conclusions

Soil oxygen and plant health characteristics measured in this research were rarely affected by NB-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. NB-oxygenated irrigation water increased the PPSO compared with untreated water in one season, but this was not observed in any other season. During overhead irrigation events, greater than 50% of total DO was lost from NB-oxygenated water because of 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 sand-based creeping bentgrass putting green with NB-oxygenated water would increase root zone oxygen concentration or increase root and shoot growth. Unless the technology improves significantly and can be supported by further research studies, it would be difficult to recommend that golf courses with sand-based creeping bentgrass putting greens invest in an NB injection system at this time. Future research should investigate the effects of NB-oxygenated irrigation water on the growth of different turfgrass species or turfgrasses being grown in more finely textured root zones. In addition, as NB-generation technology improves, different methods of NB oxygenation warrant further investigation.

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