HortScience 60(11):1942–1947. 2025. https://doi.org/10.21273/HORTSCI18855-25

Field Evaluation of the Impact of Oxygenated Nanobubble Water on Turfgrass Growth and Soil Health

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Keywords. irrigation water, oxygenated nanobubbles, soil health, turfgrass quality

Abstract. **The need to maintain high turf quality on golf courses often leads to extensive use of inputs that can have negative financial and environmental consequences. The use of oxygenated nanobubble water has been proposed to help reduce inputs by improving turfgrass growth and soil health; however, there is limited research evaluating its applications. A 5-month field study was conducted over 2 years in Johns Creek, GA, USA, to evaluate the impacts of oxygenated nanobubble water on turfgrass growth and quality as well as soil biological health. Field plots under randomized complete block design were assigned two treatments: irrigation with oxygenated nanobubble water vs. untreated water. Shoot and root growth parameters were measured regularly to evaluate turfgrass growth, whereas turfgrass quality was evaluated with digital image analysis and visual rating. Soil health was assessed by measuring microbial abundance, inorganic nitrogen, enzyme assays, and soil respiration. Average root weight showed significant treatment effect in 2022, with nanobubble treatment yielding a higher mean than control, but these results were not consistent in both years. There were no significant treatment effects on other turfgrass or soil health parameters. Overall, the use of oxygenated nanobubble water did not consistently impact turfgrass growth, turfgrass quality, or soil biological health parameters likely because either the oxygen was lost during irrigation or the oxygen did not stay in the soil long enough to have any effect.**

High-quality turfgrass is highly desirable on golf courses. Superintendents rely on extensive use of inputs to achieve it (Strandberg et al. 2012; Thompson and Kao-Kniffin 2019). The pursuit of high-quality playing conditions carries both financial and environmental costs. As such, there is a need to explore new technologies that can reduce inputs and promote sustainable solutions. One such approach can be the adoption of oxygenated nanobubble technology, which produces nano-sized oxygen-filled cavities in a liquid. One way of generating such cavities is by pushing compressed oxygen gas through a nano-sized membrane, producing nano-sized bubbles ranging from 100 million to 10 trillion per milliliter (Atkinson et al. 2019; Phan et al. 2020). Oxygenated nanobubbles have long-term stability in water and are negatively charged at the liquid-air interface (Nirmalkar et al. 2018). Some studies have reported that the use of oxygenated nanobubble water resulted in increased yield, better water-use efficiency, and better fruit quality in lettuce, cucumber, and tomatoes (Baram et al. 2022; Ouyang et al. 2021; Zhou et al. 2019); however, there are limited studies on the use of oxygenated nanobubble water in a turfgrass system, as the technology is still in its infancy (DeBoer et al. 2024; Patel et al. 2021). The impact of oxygenated nanobubble water on turfgrass soil microbial communities is unknown as well.

In soils that have been compacted or flooded, low oxygen supply or poor soil aeration restricts root growth (Huang et al. 1998). Letey et al. (1966) observed that bermudagrass root growth ceased when oxygen diffusion rates fell below 0.15 $\mu g \cdot cm^{-2} \cdot min^{-1}$. Although some turfgrass species have been found to maintain root growth down to 5% soil oxygen, results vary widely across species and growing environments (Letey et al. 1964; Van Wijk 1980). Although low soil oxygen levels are widely believed to reduce turfgrass quality, there is relatively little research exploring root oxygen requirements. In addition, it has previously been reported that roots had lower density and reduced distribution under low oxygen conditions (O'Neil and Carrow 1983). The soil oxygen level can be improved through aeration by infusing irrigation water with oxygen (Pendergast et al. 2013). The main challenge to this approach has been the short residence time of dissolved oxygen (DO) in irrigation water (Lei et al. 2016). This problem can potentially be addressed through the infusion of irrigation water with oxygenated nanobubbles that are considered to have long-term stability in water (Nirmalkar et al. 2018). Achieving improved aeration this way can result in enhanced root respiration, improved nutrient and water-use efficiencies, enhanced photosynthesis, and ultimately improved crop yield and quality (Du et al. 2018; Ouyang et al. 2021; Zhou et al. 2019).

Microorganisms play a key role in providing ecosystem functions in healthy soils (Kumar and Verma 2019; Lehman et al. 2015). Organic matter decomposition, nutrient cycling, disease suppression, and other ecosystem services in a turfgrass system are driven by soil microorganisms, which constitute the soil's biological health (Barrios 2007; Doran and Zeiss 2000; van der Heijden et al. 2008). Microbial decomposers are essential in the breakdown of organic matter into available nutrients that can be used by plants, including by turfgrasses (Barrios 2007; Steinke and Ervin 2015; van der Heijden et al. 2008). As with plant roots, microbial activity also can be limited by low oxygen level in the soil (Li et al. 2016). Using oxygenated nanobubble water has the potential to enhance the functions of microorganisms in soils due to improved aeration and can lead to reduced use of external inputs because of the provisions that come from microbial activity.

Measurements of microbial abundance and activity are commonly used to monitor changes in soil biological health (Doran and Zeiss 2000; Griffin et al. 2023). Quantifying changes in abundance of broad groups such as total bacteria and total fungi can provide insights into the overall impact of the practice on soil microorganisms (Diera et al. 2020). Similarly, soil respiration, which is the result of microbial decomposition of organic matter, is a generic indicator of microbial activity (Allison et al. 2008; Parkin et al. 2015). Soil enzyme assays, on the other hand, can be used to obtain more specific information on microbial activity in relation to nutrient cycling (Asadishad et al. 2017; Harris and Keshwani 2009). For example, enzymes such as urease and phosphatase are mainly produced by microorganisms in soil to mediate nitrogen and phosphorous cycling (Burns et al. 2013; Pascual et al. 2002).

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

Received for publication 11 Jul 2025. Accepted for publication 21 Aug 2025.
Published online 23 Sep 2025.
The study was supported by the Specialty Crop Block Grant Program of the Georgia Department Agriculture and the Georgia Golf Environment Foundation. We are grateful to Mr. Mark Hoban for providing access to field plots and general assistance with research activities. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product and does not imply its approval to the exclusion of other products or vendors that also may be suitable.
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Materials and Methods

Study site. The field trial was established in Aug 2020 on a ultradwarf 'TifEagle' bermudagrass (*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Davy) on a putting green at the Rivermont Golf Club in Johns Creek (34.00727723583316, −84.25851864591851) and monitored for two growing seasons, which were from May 25 to Sep 27 in 2021 and May 31 to Aug 30 in 2022. Weather data were collected from a nearby site run by the University of Georgia Weather Network (http://www.georgiaweather.net/). Total precipitation during the 2021 trial was 80.65 cm and 52.60 cm in 2022 (AEMN 2022). The average maximum and minimum air temperatures for 2021 were 32.7 and 20.1 °C, respectively. The average maximum and minimum air temperatures for 2022 were 33.7 and 20.07 °C.

Experimental setup. The experimental design schematic is shown in Supplemental Fig. 1. There were two treatments with four replications, resulting in a total of eight field plots. The treatments were divided by the irrigation water type. The first treatment received pond water without oxygenated nanobubbles (101, 102, 103, 104), acting as a control for the study. The second treatment received pond water with oxygenated nanobubbles (201, 202, 203, 204). The irrigation water was obtained from the pond in the golf club. Duplicate samples of the pond water were sent to the University of Georgia's Agricultural and Environmental Services Laboratory (https://aesl.ces.uga.edu/soil.html) for testing and had the following chemical properties: slightly hard water (33 $mg \cdot L^{-1}$), pH (7.4), alkalinity (36 $mg \cdot L^{-1}$), calcium (8.5 $mg \cdot L^{-1}$), chloride (6.42 $mg \cdot L^{-1}$), EC (97 µS/cm), magnesium (2.8 $mg \cdot L^{-1}$), and negligible amounts of aluminum, boron, copper, iron, and fluoride. Each plot was 1.2 m × 1.2 m, with a 0.38-m buffer between them. The plots were arranged in a randomized complete block design with four replicates. Soil oxygen and temperature sensors (Apogee SO-110 Soil Response Thermistor Reference Oxygen Sensors; Apogee Instruments, Logan, UT, USA) were placed at a depth of 10 cm (4 inches) in each plot of the putting green. Before installation, the sensors were used to take air current output (mV). The atmospheric oxygen concentration (20.95%) was divided by the atmospheric current reading to obtain the multiplier factor to convert the probe current readings in the soil to oxygen concentration in percent (Bugbee and Blonquist 2006). The field plots were on a putting green sandy soil with 3% organic matter and an average pH of 6.5. The putting green was originally built based on specifications of the US Golf Association. The organic matter was determined based on the loss-on-ignition method (Schulte and Hopkins 1996), and the pH was measured with a pH electrode (Thermo Scientific VSTAR94; Thermo Fisher Scientific, Waltham, MA, USA) in 1:1 mixture of soil:water. Plant and soil samples were taken monthly during the growing season as described in the following. The field sampling schedules are shown in Supplemental Table 1.

The nanobubbles were generated with a 50-gallon per minute Moleaer unit (Moleaer, Carson, CA, USA). The formation of nanobubbles was confirmed with measurement (Supplemental Fig. 2) using a Nanosight (Malvern Panalytical, Westborough, MA, USA). A NorthStar 98-L, 12-V sprayer was used for irrigation, delivering 20 $L \cdot min^{-1}$ from a Cool Shot Plus drenching nozzle. Irrigation treatments were applied three times per week to replace 70% reference evapotranspiration (AEMN 2022). Total DO ($mg \cdot L^{-1}$) in the irrigation water, before and after passing through spray nozzles, was recorded at each irrigation event with a DO meter (HI98193; Hannah Instruments, Woonsocket, RI, USA).

Fertilizers were applied weekly during the growing season, from April to November. The fertilizers were a combination of organic and synthetic sources, with foliar applications that provided 45.4 g to 68.0 g of nitrogen per week, using a fertilizer of 3.2N–0.14P–1.7K analysis. In addition, Daconil (a.i. = chlorothalonil, Syngenta, Basel, Switzerland) was used in one instance for cream leaf spot in the plots. The greens were maintained at 0.267 cm (0.105 inches) height during the growing season, raising the mowing height during dormant turfgrass periods for winter.

Plant analysis

A soil core cylinder with dimensions of 36.8 cm in length and 5.1 cm in width was hammered into the ground using a rubber hammer to a depth of 15 cm to collect samples. The soil core was used for analysis of turfgrass physiological parameters. After sampling, it was placed in a plastic bag that was kept in a cooler for transport to the University of Georgia Griffin campus for analysis. The soil core was dismantled to measure shoot weight, root weight, and to carry out root scan image analysis as described in the following.

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

Rooting traits analysis. After the shoot and thatch layer at a standard depth of 1 cm were removed from the core as described previously, the roots were washed thoroughly in a sieve and picked with tweezers. The samples were placed in an 80% ethanol solution until scanned. The roots were placed on a

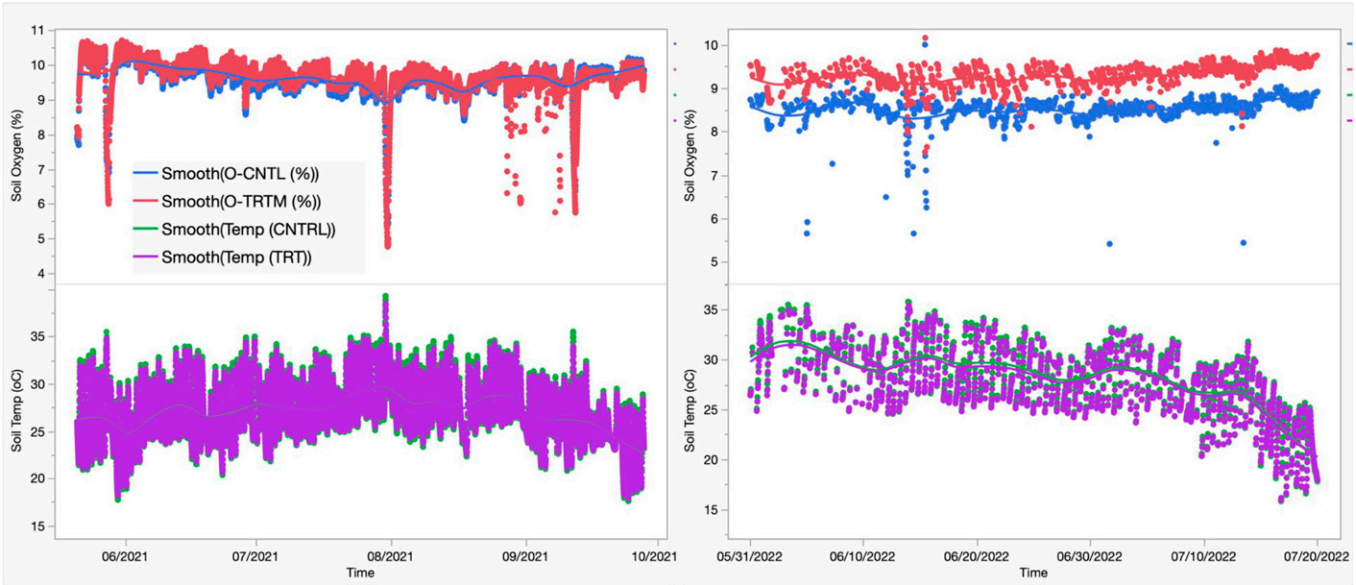

Fig. 1. Mean soil oxygen and temperature in plots under oxygenated nanobubble pond water (TRTM) or pond water (CNTRL) during the study period in 2021 and 2022.

plastic tray used for scanning through an Epson Scanner (Epson Perfection V550 Photo; Epson, Los Alamitos, CA, USA). The plastic tray was 30.48 cm in length, 21.59 cm in width, and 1.27 cm in height to fit the scanner and hold a thin layer of water. The thin layer of water was used to suspend representative roots in an aqueous solution. Debris and organic matter were removed from the root samples. The tray and root samples were scanned, producing a 600-dpi-resolution high-quality JPG image that was saved to a computer (Katuwal et al. 2020). The images were then run through the GIAroots digital imaging root analysis program (https://www.quantitative-plant.org/software/giaroots) to yield the average root width, the maximum number of roots, the median number of roots, and number of the connected component (Galkovskyi et al. 2012). After scanning, the root samples were removed from the plastic scanning tray and placed into #1-coin envelopes (Quality Park, 2.25 × 3.5 inches) and put in an incubator at 70 °C for 4 d or more to dry out. Dry weight of the roots was measured in a balance (Mettler AE 100; Mettler-Toledo).

Turfgrass quality with digital image analysis and visual rating. For digital image analysis, a Canon G9X digital camera (Canon, Tokyo, Japan) with the custom settings of Iso: 400, Flower interface, F: 4.0, and custom white balance of 0 was used. A lightbox is used to capture an image of the plot to ensure uniformity of lighting. One image was taken per plot. The images were processed through Fiji ImageJ software (National Institutes of Health, Bethesda, MD, USA) (Schindelin et al. 2015) to obtain percent green cover, which provides an objective assessment of the overall turf quality and quantitative data for statistical analysis (Jespersen et al. 2019). A visual rating was also assigned to each plot based on turfgrass quality. This is the most commonly used method for assessing turfgrass quality by superintendents. It was based on a scale of 1 to 9, using color, density, and uniformity to determine the rating, as described in Krans and Morris (2007).

Soil sampling and processing. Soil samples were taken monthly during the growing season (Supplemental Table 1). Composite soil samples of six soil cores were collected from the top 10 cm at random from each plot using a soil probe, which was 2.54 cm in diameter and 15.24 cm in depth. During sampling, the soil probe was sprayed with alcohol and wiped cleaned between each plot to prevent cross-contamination. Soil samples were stored in plastic bags and placed in a cooler with ice packs until they could be processed. Once in the laboratory, the samples were sieved through a 2-mm sieve to remove plant materials. Part of the sieved samples was then used to measure soil respiration, enzyme activities, and ammonium and nitrate concentrations. Samples were analyzed immediately or within 2 weeks of storage in a refrigerator at 4 °C. Approximately 10 g of soil from each bag was also immediately frozen at −15 °C for later DNA extraction to determine microbial abundance via quantitative polymerase chain reaction (qPCR).

Measurement of soil biological health indicators. Phosphatase activity was determined colorimetrically as described in Tabatabai (1994). Urease activity was determined with a boric acid trap method as described in Mobley and Hausinger (1989). Soil respiration was determined with the alkaline trap method as described in Zibilske (1994). For ammonium and nitrate analysis, KCl extraction from soil was conducted according to Habteselassie et al. (2006) in which a suspension of soil was prepared with a 1:5 ratio of soil and 2M KCl and shaken for 2 h, followed by filtration. The KCl extracts were sent to Waters Agricultural Laboratories (Camilla, GA, USA) to analyze for ammonium and nitrate with a flow-injection autoanalyzer. Soil moisture was determined gravimetrically in which 10 g of fresh soil was weighed (Model Adventurer Pro AV2102C; Ohaus Corp., Pine Brook, NJ, USA) in aluminum tin and dried for 24 h in an oven at 100 °C. The soil was cooled in a desiccator and weighed to obtain the oven-dry weight. Results were then expressed per oven-dry weight equivalent basis.

Quantitative polymerase chain reaction. qPCR was used to determine the abundance of total bacteria and total fungi. Using DNeasy PowerSoil DNA extraction kit (Qiagen, Germantown, MD, USA), soil DNA was extracted from samples from Jul 2021, Aug 2021, Jul 2022, and Aug 2022. The total reaction volume was 20 µL that included 10 µL of 2X PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, Grand Island, NY, USA), 2 µL template DNA, 1 µL of forward and 1 µL of reverse primers (final concentration of 250 nM), and 6.0 µL nuclease-free PCR water. All reactions were run in analytical duplicate. Further details on the primers, amplicon lengths, target genes, and reaction conditions are indicated in Supplemental Table 2. The standards were prepared through serial dilutions of stock standards for each organism and ranged from 30 to 3×10^5 copies. Standards were run in analytical triplicate for all assays. StepOne Software (Applied Biosystems, Foster City, CA, USA) was used to analyze the qPCR data. The qPCR reaction efficiencies and R^2 values for standard curves ranged from 85% to 110% and 0.95 to 0.98.

Statistical analysis. Repeated measures analysis of variance was carried out in testing the statistical significance of the effects of oxygenated nanobubble water on turfgrass and soil health parameters at a significance of 0.05 in JMP Pro 16 (SAS JMP, Cary, NC, USA). Tukey's test was used for mean separation when the treatment effect was significant.

Results and Discussion

Soil oxygen and temperature. The soil oxygen level was not significantly different between the nanobubble water and control plots ($P = 0.083$), nor was there any significant treatment × time interaction effects ($P = 1.000$). The average soil oxygen levels in the nanobubble-treated and control plots were 9.7% and 9.6% in 2021 and 8.5% and 9.3% in 2022, respectively (Fig. 1). Similarly, the soil temperature showed no significant difference between the treatment and control plots ($P = 0.3217$), nor was treatment × time interaction significant ($P = 1.000$). The average soil temperatures in the nanobubble-treated and control plots was 27 and 27 °C in 2021 and 27.7 and 27.9 °C in the control plots (Fig. 1).

Turfgrass growth and quality. There were no significant treatment effects on turfgrass growth parameters, including shoot weight, root weight, root width, numbers of roots, and interconnected root components in 2021 (Tables 1 and 2). The only difference in 2022 was that treatment effect was significant on root weight, with the nanobubble water resulting in higher root weight than the control by 26%. Similarly, there were no significant treatment effects on turfgrass quality as % green cover or with visual rating (Tables 1 and 2). The significant effect in all these parameters had to do with the sampling date (Table 1). The turfgrass quality with visual rating and % green cover was higher than 8% and 84% throughout the study, respectively (Table 2).

Microbial abundance and activity. There were no significant treatment effects on total

Table 1. Mixed model analysis of plant growth and microbial activity parameters in TifEagle bermudagrass soil treated with nanobubble or control pond water.

	Main effect P value ($\alpha = 0.05$)					
	2021			2022		
Response variables	Treatment (T)	Sampling date (SD)	T × SD	Treatment	Sampling date	T × SD
Shoot weight	0.508	<0.001	<0.001	0.473	0.123	0.992
Root weight	0.517	0.003	0.250	0.047	0.005	0.728
Mean root width	0.057	<0.001	0.134	0.153	0.180	0.937
Maximum no. of roots	0.920	0.026	0.463	0.101	<0.001	0.044
Med. no. of roots	0.813	0.016	0.507	0.283	<0.001	0.164
No. of connected root components	0.656	<0.001	0.679	0.374	0.423	0.166
Turfgrass quality (% green cover)	0.835	<0.001	0.914	0.406	<0.001	0.226
Turfgrass quality (visual rating)	0.658	<0.001	0.331	1.000	<0.001	1.000
Soil respiration	0.189	<0.001	0.027	0.121	0.023	<0.001
Urease activity	0.125	<0.001	0.905	0.673	0.006	0.002
Phosphatase activity	0.411	0.218	0.672	0.960	0.003	0.976

Med. = Median.

Table 2. Mean turfgrass growth and turfgrass quality parameters in response to treatment with oxygenated nanobubble water or non-treated water.

		Year	
Parameters	Treatment	2021	2022
Shoot weight (g)	Nanobubble water	1.76 a	2.81 a
	Control	1.66 a	2.97 a
Root weight (g)	Nanobubble water	0.090 a	0.135 a
	Control	0.097 a	0.107 b
Mean root width (cm)	Nanobubble water	0.022 a	0.027 a
	Control	0.021 a	0.026 a
Maximum no. of roots	Nanobubble water	31.9 a	28.6 a
	Control	31.6 a	25.5 a
Med. no. of roots	Nanobubble water	22.7 a	20.3 a
	Control	23.3 a	18.6 a
No. of connected root components	Nanobubble water	64.9 a	102.0 a
	Control	62.1 a	94.5 a
Turf quality (% greenness)	Nanobubble water	84.9 a	88.4 a
	Control	84.7 a	89.4 a
Turf quality (visual rating)	Nanobubble water	8.77 a	8.32 a
	Control	8.75 a	8.32 a

Means with the same letter suffixes are not significantly different from each other within a year; Med. = Median.

bacteria ($P = 0.447$) or total fungi ($P = 0.222$). The mean total bacterial abundance for control was 1.5×10^7 copies/g soil and the mean total bacteria for treatment was 1.2×10^7 copies/g soil. The mean total fungal abundance for control was 4.2×10^6 copies/g soil and the mean total fungi for treatment was 2.3×10^6 copies/g soil.

None of the microbial activity parameters (respiration and enzyme activities) were significantly impacted by the treatment in both years (Tables 1 and 3). There were significant sampling date × treatment effects for 2021 and 2022. There were no significant treatment effects on nitrate ($P = 0.279$) or ammonium concentrations ($P = 0.3044$) either. The mean ammonium concentrations for treatment and control were 9.19 and 7.60 $mg \cdot kg^{-1}$ soil, respectively. The mean nitrate concentrations for treatment and control were 1.65 and 1.57 $mg \cdot kg^{-1}$ soil, respectively.

Overall, oxygenated nanobubble water did not significantly impact turfgrass growth or quality, nor did it significantly impact biological soil health parameters (soil respiration and enzyme activities). The only significant treatment effect that was observed was on root weight in 2022. We evaluated the impact of oxygenated nanobubble water on the turfgrass and soil health in several ways that are sensitive to oxygen input into the soil. Aeration promotes root growth and water-use efficiency, increasing plant biomass (Lei et al. 2016; Pendergast et al. 2013). It also stimulates the growth and activity of the microorganisms in the root zone (Zhu et al. 2019). It promotes soil processes such as the oxidation of ammonium to nitrate (nitrification), which is an aerobic process. Plants and microbes respond to oxygen input in a manner described previously when oxygen is limiting in the soil. As such, the lack of consistent treatment effect of oxygenated nanobubble water on turfgrass growth and quality and soil biological health suggest that the system was either not limited by oxygen availability or the oxygen was not staying in the soil system on application.

Continuous monitoring of soil oxygen indicated similar levels of oxygen in both types of plots, indicating that the irrigation water with oxygenated nanobubbles did not change the oxygen concentration in the soil (Fig. 1). In this study, the soil oxygen levels were below what others had reported (Baram et al. 2021; DeBoer et al. 2024). This was unexpected in a sand-based system where aeration is commonly assumed to be good. The sensor data and the datalogger program were shared with a technical support staff member from Apogee Instruments (the maker of the sensors), and no problem was detected in sensor performance or data quality. As part of the general maintenance on the golf club, the green is watered regularly. The soil volumetric water content was above field capacity for sandy soils (Supplemental Fig. 3). It is possible that excess moisture might have led to poor aeration conditions in the plots, but no sign of plant stress was observed based on the turfgrass growth and quality parameters.

A high level of DO was achieved initially in the irrigation water (~32 $mg \cdot L^{-1}$) with the nanobubble unit; however, there was a significant decrease in DO at the nozzle during irrigation (22 $mg \cdot L^{-1}$), indicating loss of oxygen from the water during application (Table 4). It also seems highly likely that further loss of oxygen took place once the water entered the soil. The fact that the soil oxygen was low even after being irrigated with water that initially had a high level of DO suggests that oxygen in the water did not stay in the soil. This might explain the lack of response from the turfgrass and soil microorganisms even if it seemed that the soil oxygen was low.

Among the few studies in turfgrass, DeBoer et al. (2024) examined the impact of oxygenated nanobubbles in irrigation water on the turfgrass quality and growth in creeping bentgrass. Similar to our findings, nanobubble water did not consistently improve soil oxygen level in a field study over a 3-year period. There was no significant effect of nanobubble water on turfgrass quality, clipping yield, and nitrogen content or root growth in both field and greenhouse studies, even though the nanobubble unit increased the DO level in irrigation water significantly (e.g., 29 $mg \cdot L^{-1}$ vs. 9 $mg \cdot L^{-1}$ in the greenhouse study) as compared with the control water. DeBoer et al. (2024) reasoned that there was no significant response from the turfgrass as a result of oxygenated nanobubble water because oxygen was not limiting in their sand-based soil system.

Most previous studies on this topic focused on fruits and vegetables. In a study by Baram et al. (2022), irrigation with nanobubble water improved lettuce yield, root viability, and chlorophyll content. Oxygenated nanobubble water improved oxygen availability in soil. The effect was more pronounced in the poorly aerated clay soil than in the well-aerated sandy soil. In addition, in most cases subsurface application of the oxygenated nanobubble water resulted in better plant outcomes than surface application. In aggregate, their studies suggest that plants are more responsive to oxygenated nanobubble water when oxygen is limiting, and that mode of application might be important in keeping as much of the applied oxygen in the soil as possible. Overhead application of irrigation water, as

Table 3. Soil health parameters in response to treatment with oxygenated nanobubble water or non-treated water.

		Year	
Parameters	Treatment	2021	2022
Soil respiration	Nanobubble water	1.88 a	1.84 a
($mg \cdot g^{-1}$ CO_2 soil × d^{-1})	Control	1.85 a	1.85 a
Urease activity	Nanobubble water	15.69 a	6.16 a
($\mu mol \cdot g^{-1}$ NH_3 × h^{-1})	Control	16.96 a	6.44 a
Phosphatase activity	Nanobubble water	0.51 a	0.19 a
($\mu mol \cdot g^{-1}$ pNP × h^{-1})	Control	0.61 a	0.19 a
Total bacteria	Nanobubble water	7.33 a	6.59 a
(log copies/g soil)	Control	7.44 a	6.61 a
Total fungi	Nanobubble water	7.61 a	5.88 a
(log copies/g soil)	Control	6.90 a	5.79 a
Nitrate conc.	Nanobubble water	1.65 a	1.66 a
(mg NO3-N·kg^{-1} soil)	Control	1.49 a	1.65 a
Ammonium conc.	Nanobubble water	6.41 a	11.97 a
(mg NH4-N·kg^{-1} soil)	Control	4.87 a	10.32 a

Means with same letter are not significantly different at $P = 0.05$ within parameter and year; conc. = concentration.

Table 4. Mean dissolved oxygen and temperature of nanobubble treated and non-treated irrigation water before and after spray.

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

was done in our study, might not be the best way to deliver it to the soil, as it might result in loss of the DO and breakup of the oxygenated nanobubbles in the water. This might explain the decrease in DO we saw at the sprayer nozzle (Table 4). However, Baram et al. (2022) speculated that part of the lettuce response to application of oxygenated nanobubble water might have come from hydroxyl radicals that form from the nanobubbles based on their calculations of how much oxygen was delivered to the soil in dissolved form and inside nanobubbles. Unlike our study, their study was not a field study where there is less control on environmental variables that affect soil oxygen residence.

In another study in which the micro and nanobubble oxygenated irrigation water was supplied through a subsurface drip irrigation system, Zhou et al. (2019) reported improved dry matter accumulation, fruit quality, and water-use efficiency in tomato and cucumber. In addition, the treatment positively affected soil biological health by improving soil enzyme activities, including urease, phosphatase, and catalase. The composition and abundance of the soil microbial community were also responsive to the treatment. The study was done with clay loam soil in a greenhouse, and no information was provided on the aeration status of the soil either before or during the study period. Clay soils are often not well aerated. It is possible that the treatment improved the soil oxygen concentration. The study setup also might have minimized loss of oxygen from the soil as the water was applied through a subsurface drip irrigation system, and the soil was covered with plastic mulch on top. In a similar greenhouse study with a sandy loam soil in which oxygenated micro and nanobubble irrigation water was supplied via subsurface drip irrigation, Ouyang et al. (2021) reported improved photosynthesis, growth, and yield in tomatoes. In addition, they observed increases in microbial abundance and activity (soil respiration and enzyme activities) as a result of the treatment vs. nontreated soils. It was not clear what the soil oxygen status was as it was not monitored during the study period, nor was there any information on the initial soil aeration status. However, the use of subsurface drip irrigation and plastic films to cover the soil might have helped in keeping the oxygen in soil.

The potential benefits of oxygenated nanobubble water are believed to be associated with two things: increase in aeration and formation of reactive oxygen species (ROS) such as hydroxyl radicals from the collapse of oxygenated nanobubbles (Phan et al. 2020). It is well known that ROS are involved in plant growth and development (Mhamdi and Van Breusegem 2018). The process of creating oxygenated nanobubbles significantly increases the DO level in irrigation water as indicated in this report and others (DeBoer et al. 2024). It also supplies oxygen in nanobubbles. Maintaining the oxygen, especially oxygen supplied in the dissolved form, might require the use of subsurface irrigation systems that minimize its loss. The oxygen inside the nanobubbles might not be of the magnitude to significantly contribute to the soil oxygen level (Baram et al. 2022). Examining the use of oxygenated nanobubbles in irrigation water in turfgrass from these two perspectives suggests that the benefits would most likely come from the hydroxyl radicals, as irrigation is often applied via overhead systems, which might result in loss of most of the DO. However, more research is needed to understand factors that determine the residence time and stability of oxygenated nanobubbles and formation of ROS from nanobubbles in turfgrass soils under a range of aeration conditions. Our current study suggests that oxygen input into the soil did not make much difference despite low soil oxygen level and might have been lost quickly.

References Cited

AEMN. 2022. University of Georgia Weather Network. College of Agricultural and Environmental Sciences. University of Georgia. http://georgiaweather.net.

Allison SD, Czimczik CI, Treseder KK. 2008. Microbial activity and soil respiration under nitrogen addition in Alaskan boreal forest. Glob Change Biol. 14(5):1156–1168. https://doi.org/10.1111/j.1365-2486.2008.01549.x.

Asadishad B, Chahal S, Cianciarelli V, Zhou K, Tufenkji N. 2017. Effect of gold nanoparticles on extracellular nutrient-cycling enzyme activity and bacterial community in soil slurries: Role of nanoparticle size and surface coating. Environ Sci: Nano. 4:907–918. https://doi.org/10.1039/C6EN00567E.

Atkinson A, Apul OG, Schneider O, Garcia-Segura S, Westerhoff P. 2019. Nanobubble technology offers opportunities to improve water treatment. Acc Chem Res. 52(5):1196–1205.

Baram S, Evans JF, Berezkin A, Ben-Hur M. 2021. Irrigation with treated wastewater containing nanobubbles to aerate soils and reduce nitrous oxide emissions. J Clean Prod. 280:124509. https://doi.org/10.1016/j.jclepro.2020.124509.

Baram S, Weinstein M, Evans JF, Berezkin A, Sade Y, Ben-Hur M, Bernstein N, Mamane H. 2022. Drip irrigation with nanobubble oxygenated treated wastewater improves soil aeration. Sci Hortic. 291:110550. https://doi.org/10.1016/j.scienta.2021.110550.

Barrios E. 2007. Soil biota, ecosystem services and land productivity. Ecol Econ. 64(2):269–285. https://doi.org/10.1016/j.ecolecon.2007.03.004.

Bugbee B, Blonquist M. 2006. Absolute and relative gas concentration: Understanding oxygen in air. https://www.apogeeinstruments.com/oxygensensor_techinfo.htm. [accessed 18 Jun 2025].

Burns RG, DeForest JL, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MN, Zoppini A. 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biol Biochem. 58:216–234. https://doi.org/10.1016/j.soilbio.2012.11.009.

DeBoer EJ, Richardson MD, McCalla JH. 2024. Irrigation of sand-based creeping bentgrass putting greens with nanobubble-oxygenated water. HortTechnology. 34(1):60–70. https://doi.org/10.21273/HORTTECH05322-23.

Diera AA, Raymer PL, Martinez-Espinoza AD, Bauske E, Habteselassie MY. 2020. Evaluating the impact of turf-care products on soil biological health. J Environ Qual. 49(4):858–868. https://doi.org/10.1002/jeq2.20080.

Doran JW, Zeiss MR. 2000. Soil health and sustainability: Managing the biotic component of soil quality. Appl Soil Ecol. 15(1):3–11. https://doi.org/10.1016/S0929-1393(00)00067-6.

Du YD, Niu WQ, Gu XB, Zhang Q, Cui BJ, Zhao Y. 2018. Crop yield and water use efficiency under aerated irrigation: A meta-analysis. Agric Water Manage. 210:158–164. https://doi.org/10.1016/j.agwat.2018.07.038.

Galkovskyi T, Mileyko Y, Bucksch A, Moore B, Symonova O, Price CA, Topp CN, Iyer-Pascuzzi AS, Zurek PR, Fang S, Harer J, Benfey PN, Weitz JS. 2012. GiA Roots: Software for the high throughput analysis of plant root system architecture. BMC Plant Biol. 12:116. https://doi.org/10.1186/1471-2229-12-116.

Griffin W, Habteselassie M, Martinez-Espinoza AD, Raymer P, Sintim H, Pennisi S. 2023. Evaluating the impact of two biological products on soil biological health and turfgrass quality. Water Air Soil Pollut. 234(10):656–666.

Habteselassie MY, Miller BE, Thacker SG, Stark JM, Norton JM. 2006. Soil nitrogen and nutrient dynamics after repeated application of treated dairy-waste. Soil Sci Soc Am J. 70(4):1328–1337. https://doi.org/10.2136/sssaj2005.0189.

Harris TK, Keshwani MM. 2009. Measurement of enzyme activity. Methods Enzymol. 463:57–71. https://doi.org/10.1016/S0076-6879(09)63007-X.

Huang B, Liu X, Fry JD. 1998. Effects of high temperature and poor soil aeration on root growth and viability of creeping bentgrass. Crop Sci. 38(6):1618–1622. https://doi.org/10.2135/cropsci1998.0011183X003800060034x.

Jespersen D, Leclerc M, Zhang G, Raymer P. 2019. Drought performance and physiological responses of bermudagrass and seashore paspalum. Crop Sci. 59(2):778–786. https://doi.org/10.2135/cropsci2018.07.0434.

Katuwal KB, Schwartz B, Jespersen D. 2020. Desiccation avoidance and drought tolerance strategies in bermudagrasses. Environ Exp Bot. 171:103947. https://doi.org/10.1016/j.envexpbot.2019.103947.

Krans JV, Morris K. 2007. Determining a profile of protocols and standards used in the visual field assessment of turfgrasses: A survey of national turfgrass evaluation program-sponsored university

scientists. Appl Turfgrass Sci. 4(1):1–6. https://doi.org/10.1094/ats-2007-1130-01-tt.

Kumar A, Verma JP. 2019. The role of microbes to improve crop productivity and soil health, p 249–265. In: Achal V, Mukherjee A (eds). Ecological wisdom inspired restoration engineering. EcoWISE. Springer, Singapore. https://doi.org/10.1007/978-981-13-0149-0_14.

Lehman R, Cambardella C, Stott D, Acosta-Martinez V, Manter D, Buyer J, Maul J, Smith J, Collins H, Halvorson J, Kremer R, Lundgren J, Ducey T, Jin V, Karlen D. 2015. Understanding and enhancing soil biological health: The solution for reversing soil degradation. Sustainability. 7(1):988–1027. https://doi.org/10.3390/su7010988.

Letey J, Stolzy LH, Lunt OR, Youngner VB. 1964. Growth and nutrient uptake of newport bluegrass as affected by soil oxygen. Plant Soil. 20(2):143–148. https://doi.org/10.1007/BF01376444.

Letey L, Morgan WC, Richards SJ, Valoras N. 1966. Physical soil amendments, soil compaction, irrigation, and wetting agents in turfgrass management iii. effects on oxygen diffusion rate and root growth. Agron J. 58(5):531–535. https://doi.org/10.2134/agronj1966.00021962005800050024x.

Lei H, Bhattarai S, Balsys R, Midmore DJ, Holmes T, Zimmerman W. 2016. Temporal and spatial dimension of dissolved oxygen saturation with fluidic oscillator and Mazzei air injector in soilless irrigation systems. Irrig Sci. 34(6):421–430. https://doi.org/10.1007/s00271-016-0512-x.

Li Y, Niu W, Wang J, Liu L, Zhang M, Xu J. 2016. Effects of artificial soil aeration volume and frequency on soil enzyme activity and microbial abundance when cultivating greenhouse tomato. Soil Sci Soc Am J. 80(5):1208–1221. https://doi.org/10.2136/sssaj2016.06.0164.

Mhamdi A, Van Breusegem F. 2018. Reactive oxygen species in plant development. Development. 145(15):dev164376. https://doi.org/10.1242/dev.164376.

Mobley HL, Hausinger RP. 1989. Microbial ureases: Significance, regulation, and molecular characterization. Microbiol Rev. 53(1):85–108. https://doi.org/10.1128/mr.53.1.85-108.1989.

Nirmalkar N, Pacek AW, Barigou M. 2018. On the existence and stability of bulk nanobubbles. Langmuir. 34(37):10964–10973. https://doi.org/10.1021/acs.langmuir.8b01163.

O'Neil KJ, Carrow RN. 1983. Perennial ryegrass growth, water use, and soil aeration status under soil compaction. Agron J. 75(2):177–180. https://doi.org/10.2134/agronj1983.00021962007500020005x.

Ouyang Z, Tian J, Yan X, Shen H. 2021. Effects of different concentrations of dissolved oxygen on the growth, photosynthesis, yield and quality of greenhouse tomatoes and changes in soil microorganisms. Agric Water Manage. 245:106579. https://doi.org/10.1016/j.agwat.2020.106579.

Pascual JA, Moreno JL, Hernández T, García C. 2002. Persistence of immobilised and total urease and phosphatase activities in a soil amended with organic wastes. Bioresour Technol. 82(1):73–78. https://doi.org/10.1016/s0960-8524(01)00127-4.

Patel AK, Singhania RR, Chen C-W, Tseng Y-S, Kuo C-H, Wu C-H, Dong CD. 2021. Advances in micro- and nano bubbles technology for application in biochemical processes. Environ Technol Innovation. 23:101729. https://doi.org/10.1016/j.eti.2021.101729.

Parkin TB, Doran JW, Franco-Vizcaíno E. 2015. Field and laboratory tests of soil respiration, p 231–245. In: Doran JW, Jones AJ (eds). Methods for Assessing Soil Quality. Vol. 49. Soil Science Society of America, Madison, WI, USA. https://doi.org/10.2136/sssaspecpub49.c14.

Pendergast L, Bhattarai SP, Midmore DJ. 2013. Benefits of oxygenation of subsurface drip-irrigation water for cotton in a vertisol. Crop Pasture Sci. 64(12):1171–1181. https://doi.org/10.1071/CP13348.

Phan KKT, Truong T, Wang Y, Bhandari B. 2020. Nanobubbles: Fundamental characteristics and applications in food processing. Trends Food Sci Technol. 95:118–130. https://doi.org/10.1016/j.tifs.2019.11.019.

Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. 2015. The ImageJ ecosystem: An open platform for biomedical image analysis. Mol Reprod Dev. 82(7-8):518–529. https://doi.org/10.1002/mrd.22489.

Schulte EE, Hopkins BG. 1996. Estimation of soil organic matter by weight loss-on-ignition, p 21–31. In: Magdoff FR, Tabatabai MA, Hanlon EA (eds). Soil organic matter: Analysis and interpretation. Soil Science Society of America, Madison, WI, USA.

Steinke K, Ervin EH. 2015. Turfgrass ecology, p 347–381. In: Stier JC, Horgan BP, Bonos SA. Turfgrass: Biology, Use, and Management. Vol. 56. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, USA. https://doi.org/10.2134/agronmonogr56.c10.

Strandberg M, Blombäck K, Jensen AD, Knox JW. 2012. Priorities for sustainable turfgrass management: A research and industry perspective. Acta Agric Scandinavica, Section B-Soil Plant Sci. 62(S1):3–9. https://doi.org/10.1080/09064710.2012.682163.

Tabatabai MA. 1994. Soil enzymes, p 775–834. In: Weaver RW, Angle JS, Bottomley PS (eds). Methods of soil analysis, part 2 – microbiological and biochemical properties. Soil Science Society of America, Inc., Madison, WI, USA.

Thompson GL, Kao-Kniffin J. 2019. Urban grassland management implications for soil C and N dynamics: A microbial perspective. Front Ecol Evol. 7:1–15. https://doi.org/10.3389/fevo.2019.00315.

Van Wijk ALM. 1980. A soil technological study on effectuating and maintaining adequate playing conditions of grass sports fields. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands.

Van Der Heijden MGA, Bardgett RD, Van Straalen NM. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett. 11(3):296–310. https://doi.org/10.1111/j.1461-0248.2007.01139.x.

Zhou Y, Zhou B, Xu F, Muhammad T, Li Y. 2019. Appropriate dissolved oxygen concentration and application stage of micro-nano bubble water oxygation in greenhouse crop plantation. Agric Water Manage. 223:105713. https://doi.org/10.1016/j.agwat.2019.105713.

Zhu Y, Cai H, Song L, Chen H. 2019. Aerated irrigation promotes soil respiration and microorganism abundance around tomato rhizosphere. Soil Sci Soc Am J. 83(5):1343–1355. https://doi.org/10.2136/sssaj2018.08.0299.

Zibilske LM. 1994. Carbon mineralization, p 835–906. In: Weaver RW, Angle JS, Bottomley PS (eds). Methods of soil analysis, part 2 – Microbiological and biochemical properties. Soil Science Society of America, Inc., Madison, WI, USA.