Updates on exploratory studies of applications of nanobubbles in turfgrass

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A. INTRODUCTION

Nanobubbles are pockets of gas filled cavities that can be found attached on a surface or dispersed in liquid and hence are referred to as surface or bulk nanobubbles. Because of their small size (below 1000 nm - a millionth of a meter), they have a large surface area per unit volume, with a corresponding concentration that can get as high as hundred million to ten trillion bubbles per milliliter of liquid (Atkinson et al., 2019). Nanobubbles are negatively charged in the pH range that is common in the environment (2 to 12) (Temesgen et al., 2017). They are stable in liquid for an extended period of time, in some cases up to several weeks (Atkinson et al., 2019). Nanobubbles are generated by using different techniques, including creating pressure difference below a certain critical value that promotes cavitation (cavity formation). Examples of other techniques include sonication, electrolysis and use of membranes to push through gases of certain sizes into a flowing liquid (Phan et al, 2020). This way, several gases have been used to make nanobubbles, oxygenated nanobubbles being the most common ones. The rupture/collapse of oxygenated nanobubbles have been shown to create reactive oxygen species (ROS) such as peroxides and hydroxyl radicals (Atkinson et al., 2019). Infusion of oxygenated nanobubbles into irrigation water can have a number of potential applications in turfgrass system.

One such application is the potential to improve aeration in turfgrass root system. For instance, use of aerated irrigation water for cotton production was shown to increase soil oxygen level by two-fold (Pendergast et al., 2013). A better level of oxygenation can probably be achieved through the use of oxygenated nanobubbles because of their stability and density. This can be crucial in situations where oxygen is limiting in turfgrass (right after irrigation, due to compaction or excessive thatch layer) and can result in improved root performance, water use efficiency and biomass (Lei et al., 2016; Pendergast et al., 2013). Oxygenation can also stimulate the activity of the microorganisms in the rootzone (Zhu et al., 2019), with the potential to promote decomposition of organic matter, which is needed for controlling excessive

accumulation of thatch layer in the long-run, and mineralization of nutrients that can be used by the turf.

Another potential application of nanobubble oxygenated water in turfgrass is their use for pathogen control and pond water treatment. The production of ROS, which have high oxidizing potential, has been shown to have antimicrobial effect (Liang et al., 2019; Sumikura et al, 2007). As such, oxygenated nanobubbles can potentially play an important role in controlling common turfgrass diseases such as dollar spot, leaf spot and others. However, it is also important to note that ROS can equally be detrimental to the turfgrass soil microbial communities that carryout important functions such as nutrient cycling, organic matter decomposition (thatch control) and disease suppression (Schlatter et al., 2017; Myrold and Bottomley, 2008). It has been indicated that the impact of ROS on living cells is dependent on their concentration. While the potential benefits of nanobubble oxygenated water in turfgrass systems is explored, it is imperative to evaluate its impact on the soil microbial communities that are key for the long-term sustainability of the system.

Another application is the potential impact of nanobubbles on water movement and retention in the soil because of their effect on surface tension. Nanobubbles have been reported to act as bridging agents in bringing together hydrophobic surfaces by influencing surface forces (Alheshibri et al., 2016). Can this mean that they can help reduce water repellency in turfgrass soils caused by hydrophobic surfaces? If so, it can have a significant implication as it can improve water availability but also reduce the use of expensive chemicals (wetting agents) that are used in summertime to fight localized dry spots. Additionally, there are reports that nanobubble oxygenated water has the potential to influence water use rates in plants, which may further influence irrigation practices with nanobubble water (Liu et al., 2019).

Interest in nanobubble technology seems to be increasing rapidly in different sectors. Existing research mainly explored its potential in wastewater treatment, food processing and crop production albeit to limited extent (Phan et al., 2020; Atkinson 2019). Therefore, there is a huge need for research in its potential application in turfgrass. However, it is important to acknowledge that the technology is still in its infancy.

In this report, we present the findings of the exploratory studies we have been doing in GA since August 2020 to examine the impact of oxygenated nanobubbles on pathogen development, turf quality and growth as well as soil biological health. The objectives of the exploratory studies are listed below.

B. OBJECTIVES

The objectives of the study are to:

1. Determine the impact of irrigation water with oxygenated nanobubbles on turfgrass pathogen development *in vitro.*
2. Determine the impact of irrigation water with oxygenated nanobubbles on water movement and water use in turfgrass in greenhouse studies.
3. Determine the impact of irrigation water with oxygenated nanobubbles on turf quality and root growth in greenhouse and field studies.
4. Determine the impact of irrigation water with oxygenated nanobubbles on activity and abundance of soil microorganisms.

C. MATERIALS AND METHODS

Objective 1 (laboratory studies): Pathogen development

Two laboratory studies were conducted to examine the impact of oxygenated nanobubbles on pathogen development. In the first experiment, a seven-day old isolate of *Clareeridia monteithiana (C. monteithiana)* was grown at 23oC in 50 mL liquid broth supplemented with filtered nanobubble water or 50 mL of sterile deionized water. The experiment treatments (Nanobubble vs Water) were replicated six times. The wet weights of the fungal cultures were measured after 5 days and dried out for 2 days at room temperature to assess the dry weights. *C. monteithiana* is the causal agent of dollar spot and was obtained from seashore paspalum in 2019 at the Griffin UGA campus.

In the second experiment, a seven-day old isolate of *C. monteithiana* was grown on solid agar medium (potato dextrose agar) at 23^{o}C and was sprayed with 1 mL of filtered nanobubbles or 1 mL of sterile deionized water or not sprayed at all. The three treatments (Nanobubble, Water and Control) were replicated eight times. Mycelial growth was measured for each replication and each treatment every day for 4 days. For both experiments, statistical analysis was carried out to test significance of the impact of the treatments on mycelial growth by time with R version 4.0.4 at 95% confidence level.

Objectives 2 & 3 (greenhouse studies): *Water movement, water use and turf growth study*

Turf plugs (10 cm diameter, 15 cm deep) were taken from a mature TifEagle green on the UGA Griffin campus and transplanted into pots (10 cm diameter, 38 cm deep). Pots were then irrigated with nanobubble oxygenated water, or regular non-nanobubble water (5 replicates per treatment). Measurements included water use determined by gravimetric measurement of evapotranspiration (ET), digital image analysis (DIA) to estimate percent green cover, infiltration rates, and clipping yield.

Objectives 3 (field study): *Turf quality and root growth study*

Field plots were established on ultradwarf TifEagle bermudagrass at the Rivermont Golf Club in Johns Creek in August 2020. Standard management practices were followed for maintaining the turf plots. Each plot is 4' by 4', with 15" buffer between them (see Figure 1). We have two treatments with four replications, resulting in a total of 8 field plots. In the first treatment, the plots received irrigation water with oxygenated nanobubbles. In the second treatment, the plots received irrigation water with no nanobubbles (control).

Nanobubbles were generated with a 50 gallon per minute Moleaer unit (Moleaer, Carson, CA) unit. A NorthStar 98-L, 12-volt sprayer was used for irrigation, delivering 20 L min^{-1} from a Cool Shot Plus drenching nozzle. Irrigation treatments were applied 3x wk^{-1} to replace 70% reference evapotranspiration. Total dissolved oxygen (DO, mg L^{-1}) in the irrigation water, before and after passing through spray nozzles, were recorded at each irrigation event with a DO meter (HI98193, Hannah Instruments). Soil oxygen (and temperature) sensors were installed in each plot at a depth of 10 cm (4") using Apogee SO-110 Soil Response Thermistor Reference Oxygen Sensors (Apogee Instruments, Logan, UT). Soil moisture readings were also taken with a hand-held TDR probe before and after irrigation.

Turf quality, including color and density, were evaluated using DIA. Samples were collected from each plot (5 cm diameter, 15 cm deep) for analysis of above ground and below ground tissues. Root samples were collected and washed and imaged to obtain rooting traits. Subsequently, roots were oven dried for at least 72 hr at 80 °C and weighed to obtain biomass. Similarly, above ground tissue were also wash and oven dried to determine total above ground biomass.

Objective 4 (field study): *Soil biological health parameters*

Soil samples were collected from the top 10 cm of the plots for analysis of soil biological health parameters. These included soil respiration and enzyme activities (urease, phosphatase), which are considered as indicators of microbial activity and function. Soil respiration was determined in the laboratory with an incubation set-up with alkaline trap followed by titration as described in Zibilske (1994). The enzyme activities were measured following standard protocols described Tabatabai (1994) and Wallestein and Weintraub (2008). Microbial genomic DNA were also extracted for later analysis of microbial abundance of target total bacteria, total fungi and some key groups of organisms that mediate ecologically important functions.

D. RESULTS AND DISCUSSION

Nanobubble impact on pathogen development

In the first experiment (with liquid culture), there was no significant differences in the dollar spot wet weight and dry weight between the Nanobubble and Water treatments (Figure 2). In this experiment, the oxygen concentration was 21.79 mg L^{-1} and 8.67 mg L^{-1} in the filtered nanobubble water and the deionized water, respectively.

In the second experiment (with solid medium), treatment effect was significant on day 1 and day 4 of incubation but not on the other days (Figures 3 & 4). On day 1, dollar spot mycelial growth in the Nanobubble treatment was 18% and 16.7% significantly lower than the mycelial growth recorded in the Water treatment and the Control treatment, respectively (Figure 3A). On day 4, dollar spot mycelial growth in the Nanobubble treatment was significantly lower than the mycelial growth recorded in the Control treatment (Figure 3B). Because a similar mycelial growth was observed in the Water and Nanobubble treatment at day 4, the reduction of the mycelial growth under the Nanobubble treatment cannot be totally due to the presence of nanobubbles. The part of the nanobubble in the reduction of the dollar spot mycelial growth was estimated to be 2.7% on day 4 (compared to the part of the water estimated at 9.4%). In this experiment, the oxygen concentration was 21.79 mg L^{-1} and 8.67 mg L^{-1} for the filtered nanobubble water and the deionized water, respectively.

Nanobubble impact on water movement, water use and turf growth:

Results from growth chamber study in greenhouse were inconclusive. No significant differences were seen among treatments for canopy percent green cover, infiltration rates, or evapotranspiration rates (Figure 5). Similarly, under field conditions no differences were detected between oxygenated-nanobubble water and the non-treated control for either overall visual performance as measured by digital image analysis, or growth as determined by above ground or below ground biomass measurements (Figure 6).

Soil biological health:

Irrigation water with nanobubbles did not significantly affect soil health parameters (Figure 7). Soil respiration, urease and phosphatase activities were not statistically different in the plots that received irrigation water with oxygenated nanobubbles or regular water. This is contrary to what was expected. Irrigation water with nanobubbles was expected to boost the soil oxygen level, resulting in increased soil microbial activity. While irrigation water treated with oxygenated nanobubbles had significantly higher dissolved oxygen level than regular water out of the sprayer nozzle (32 mg L^{-1} vs 5 mg L^{-1}), it did not seem to have resulted into an increase in soil oxygen level in the soil. Both set of plots had similar levels of soil oxygen, ~21 ppm, which is similar to the concentration in the air (Figure 8). This suggests that the oxygen was not staying in the soil and might not have been delivered inside nanobubbles.

Equipment malfunctioning:

It is worth noting that after the initiation of the experiments it was discovered that the nanobubble generators were not functioning properly and did not seem to be producing the nanobubbles needed in order to be able to properly test their effects. Due to the lack of nanobubbles, it may be expected that there were no differences between treatments. This unfortunately largely invalidates the past several months of data collection and requires experiments to be repeated for the greenhouse and field studies. The research team at UGA has been supplied with a new model of nanobubble generator, which has already been tested to confirm the level of nanobubble generation with an instrument called Nanosight. Subsequently, the laboratory experiments on pathogen development were repeated with the new unit. The data presented in this report for the pathogen study are based on the new, properly functioning unit. We are currently preparing to repeat the greenhouse and field studies with the properly

functioning unit as well. Plants have been transplanted and re-established in the greenhouse for controlled environment testing which will commence this spring, and field trials will also resume after spring green-up with a nanobubble-generator that that was repaired (new block) at the Rivermont Golf Club.

E. SUMMARY AND CONCLUSIONS

- In a laboratory study, the growth of *C. monteithiana* (a causal agent of dollar spot) was significantly reduced (by as much as 18%) when treated with water that had oxygenated nanobubbles.
- The use of water with oxygenated-nanobubbles did not significantly change water movement, water retention, turf quality, turf growth or soil biological health in greenhouse and field studies.
- While treatment with oxygen nanobubbles increased dissolved oxygen in irrigation water, it did not translate into an increase in oxygen level in the turfgrass soil.
- The absence of nanobubble effect in the greenhouse and field studies might be due to the malfunctioning of nanobubble generators used in the studies.
- The pathogen study was repeated with a new nanobubble generator that was secured for the UGA team. The reported results are from the repeated study with the new generator.
- The nanobubbler unit at the Rivermont Golf Club was also repaired for the upcoming field study.
- The UGA team in collaboration with the Rivermont Golf Club is ready to commence the greenhouse and field studies in spring.

Figure 1. Field plot design at the Rivermont Golf Club in Johns Creek, GA. They were established in August 2020.

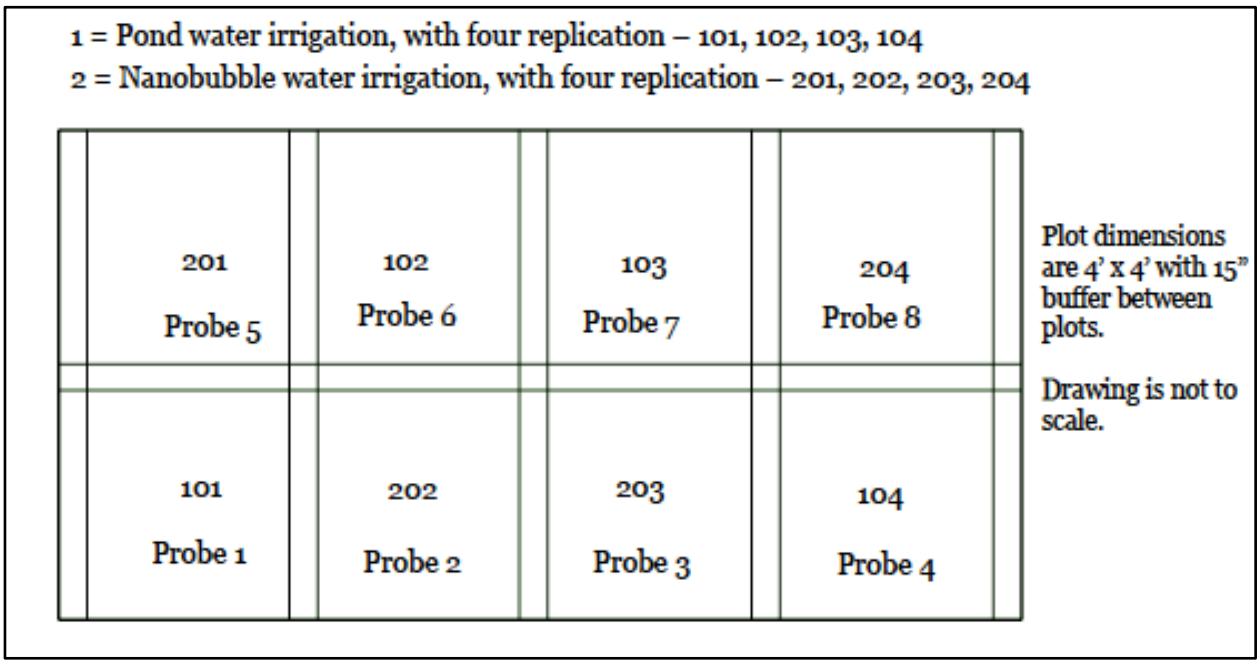


Figure 2: Wet weight and dry weight of dollar spot fungus grown in liquid culture and treated with nanobubbles or deionized water

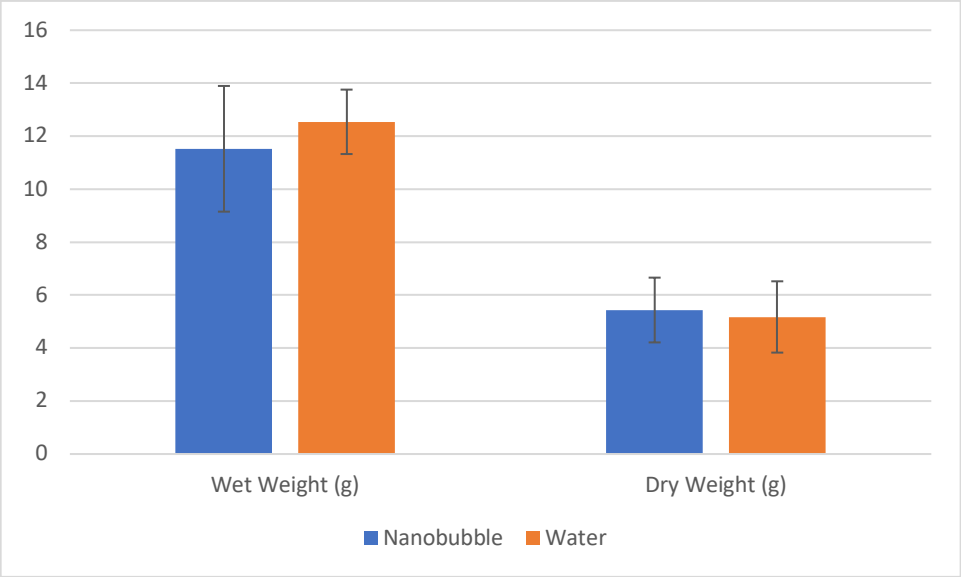


Figure 3: Dollar spot mycelial growth on solid culture medium (PDA) treated with nanobubbles, deionized water or control (no treatment) after 1 day (A) and 4 days (B) of incubation

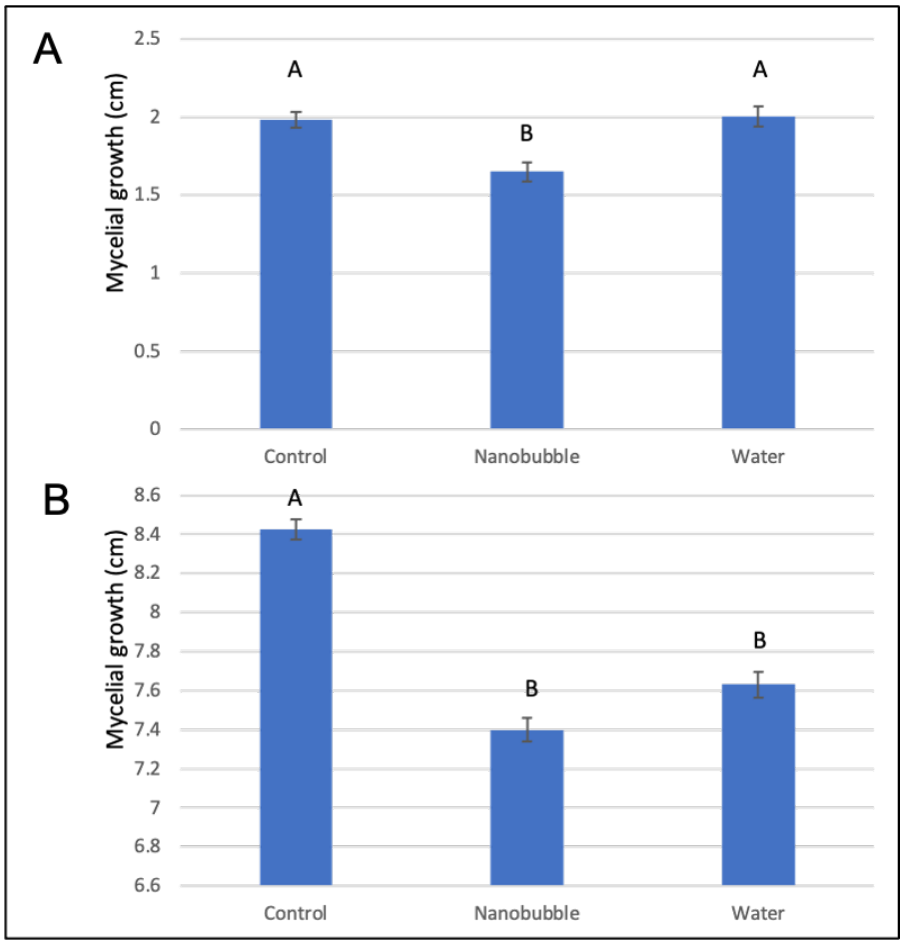


Figure 4: Mycelial growth of dollar spot fungus on solid agar medium (PDA) for the Nanobubble treatment (bottom), the Water treatment (middle) and the Control (top) after 4 days of incubation

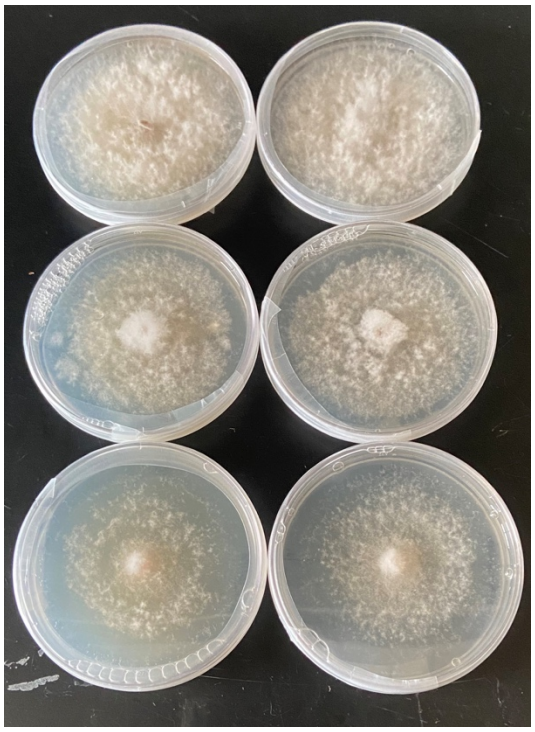


Figure 5: Percent green cover (A), infiltration rates (B), and evapotranspiration rates (C) measured during the Fall 2020 greenhouse experiments

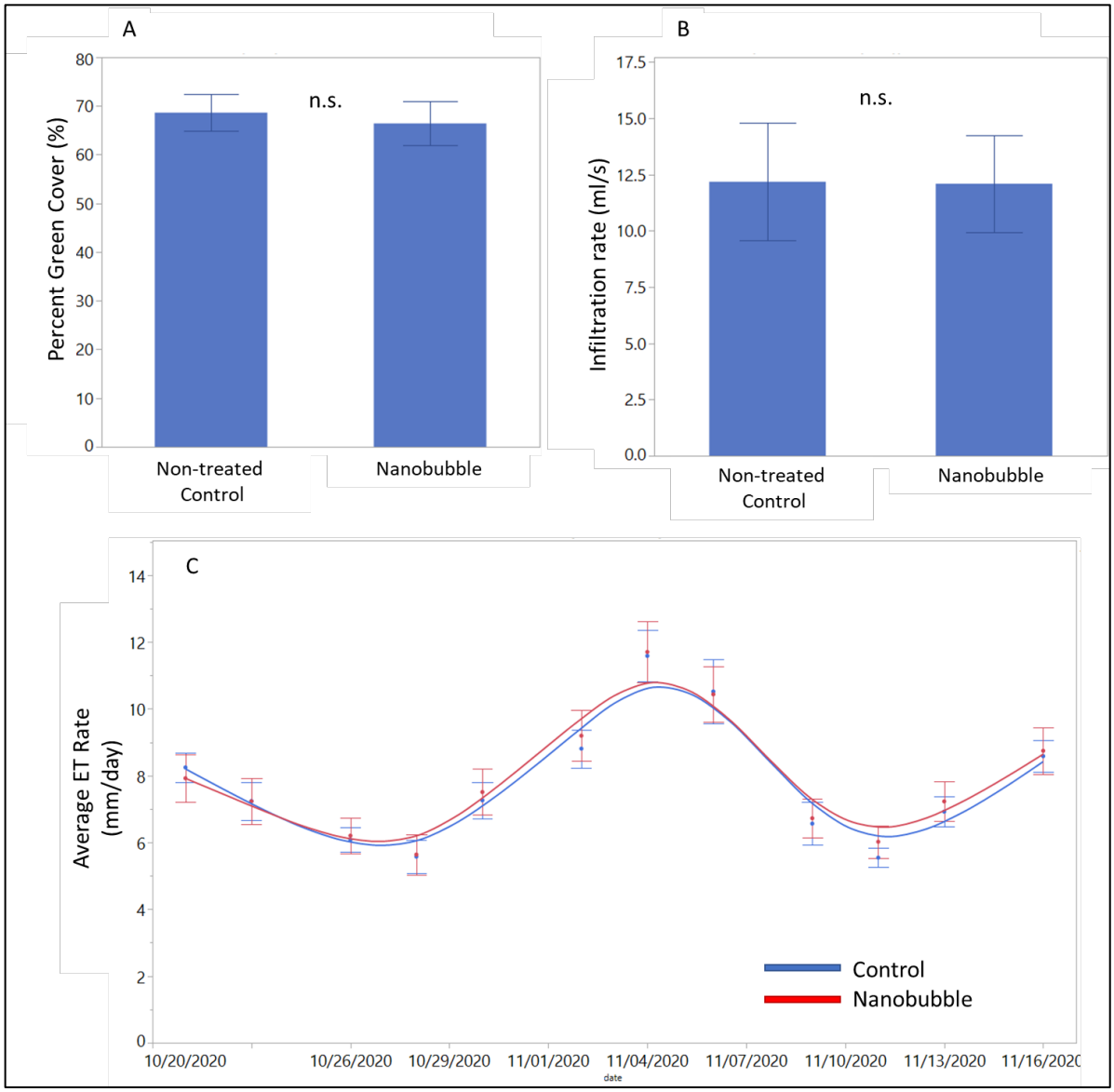


Figure 6: Percent green cover as measured by digital image analysis (A), and biomass of roots (B) and above-ground tissues (C), collected during October of the 2020 field trial in Rivermont.

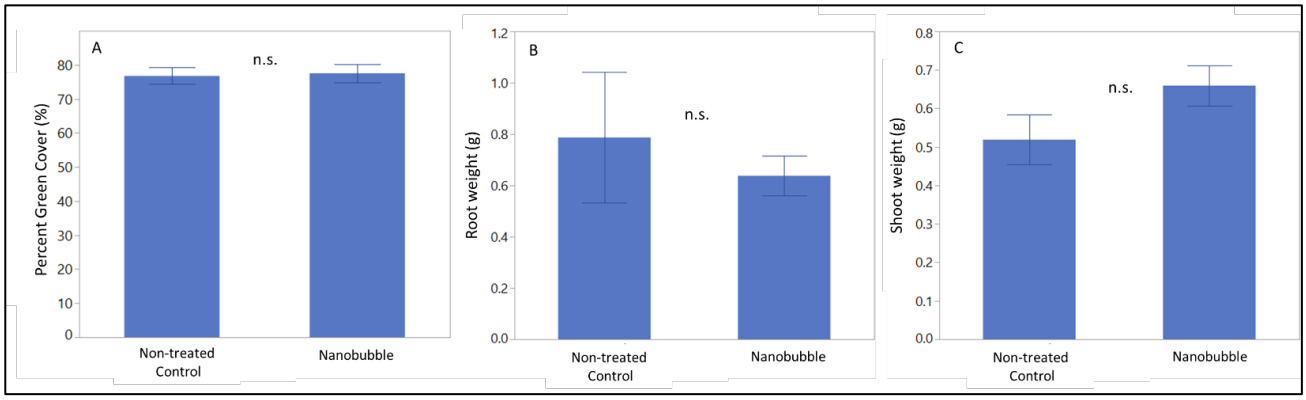


Figure 7: Mean soil respiration (mg CO_2 g^{-1} soil d^{-1}), urease (µmol NH_3 g^{-1} soil d^{-1}) and phosphatase (µmol P g^{-1} soil d^{-1}) activities in plots receiving irrigation water with or without nanobubbles on 3rd of October, November and December, 2020.

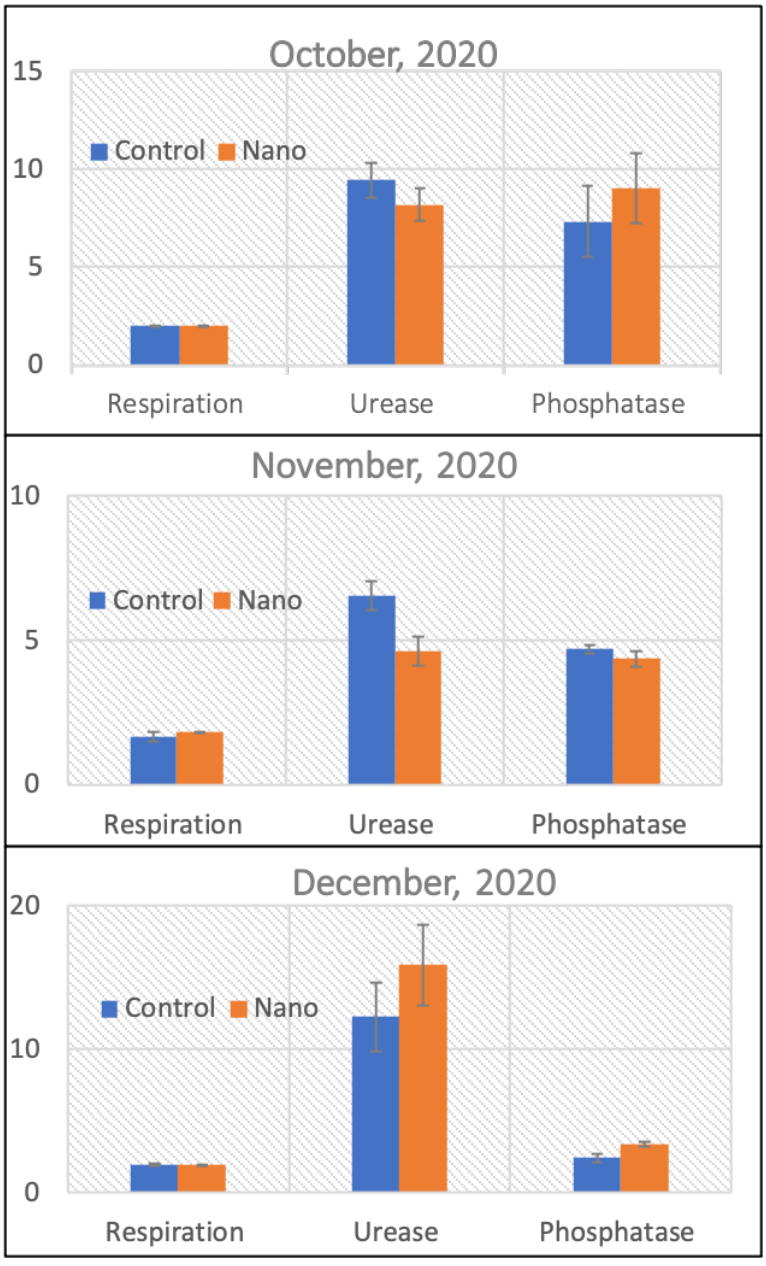
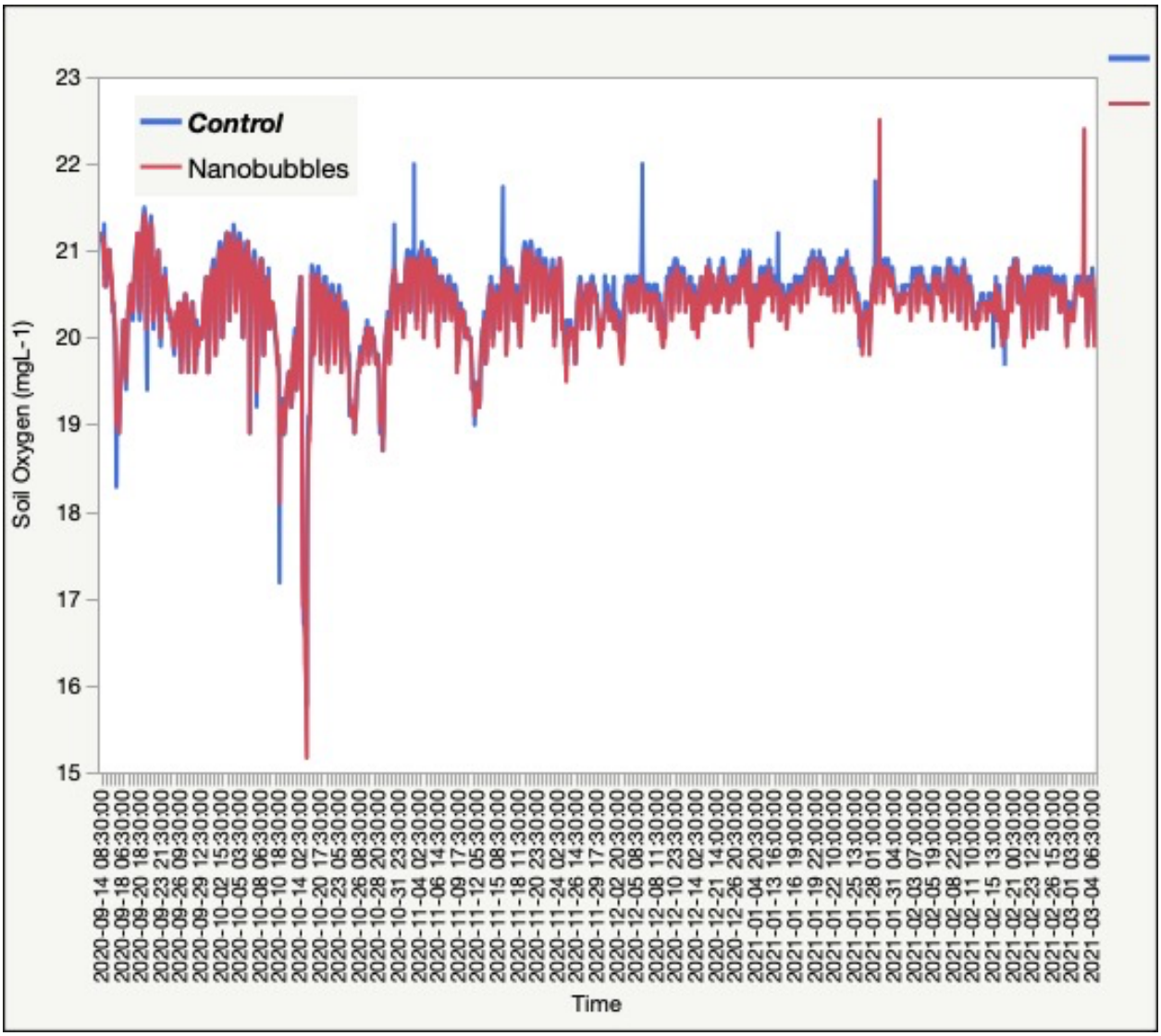


Figure 8: Mean soil oxygen levels in plots receiving irrigation water with (Nano) or without (Control) nanobubbles between Sept 14, 2020 and March 4, 2021.



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