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Research article

Electric field-based air nanobubbles (EF-ANBs) irrigation on efficient crop cultivation with reduced fertilizer dependency

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A R T I C L E I N F O

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A B S T R A C T

The advent of air nanobubbles (ANBs) has opened up a wide range of commercial applications spanning industries including wastewater treatment, food processing, biomedical engineering, and agriculture. The implementation of electric field-based air nanobubbles (EF-ANBs) irrigation presents a promising approach to enhance agricultural crop efficiency, concurrently promoting environmentally sustainable practices through reducing fertilizer usage. This study investigated the impact of EF-ANBs on the germination and overall growth of agricultural crops in soil. Results indicate a substantial enhancement in both germination rates and plant growth upon the application of EF-ANBs. Notably, the introduction of ANBs led to a significant enhancement in the germination rate of lettuce and basil, increasing from approximately 20% to 96% and from 16% to 53%, respectively over two days. Moreover, the presence of EF-ANBs facilitates superior hypocotyl elongation, exhibiting a 2.8- and a 1.6-fold increase in the elongation of lettuce and basil, respectively, over a six-day observation period. The enriched oxygen levels within the air nanobubbles expedite aerobic respiration, amplifying electron leakage from the electron transport chain (ETC) and resulting in heightened reactive oxygen species (ROS) production, playing a pivotal role in stimulating growth signaling. Furthermore, the application of EF-ANBs in irrigation surpasses the impact of traditional fertilizers, demonstrating a robust catalytic effect on the shoot, stem, and root length, as well as the leaf count of lettuce plants. Considering these parameters, a single fertilizer treatment (at various concentrations) during EF-ANBs administration, demonstrates superior plant growth compared to regular water combined with fertilizer. The findings underscore the synergistic interaction between aerobic respiration and the generation of ROS in promoting plant growth, particularly in the context of reduced fertilizer levels facilitated by the presence of EF-ANBs. This promising correlation holds significant potential in establishing more sustainability for ever-increasing environmentally conscious agriculture.

1. Introduction

Escalating the global population, coupled with persistent challenges engendered by climate change, and dwindling natural resources, has heightened the urgency to revolutionize agricultural practices more sustainably (Arora, 2019). The intensifying utilization of fertilizers in crop production (Zhang et al., 2015), to address, at least in part, these challenges, must overcome the separate and ongoing and perennial challenge concerning their limited uptake by plants (Swaney and Howarth, 2019; Bai et al., 2020). Notably, the nitrogen fertilizer's recovery ratio in harvested crops can drop to as low as 40–50%, leading to the accumulation of residual fertilizers in the soil and subsequent increases in surface runoff (Sun et al., 2012), which leads to further problems in the development of problems in runoff water that may accumulate in water bodies, e.g., cyanobacteria. Furthermore, possible traces of heavy metals due to the consumption of fertilizer can negatively influence the ecosystem and water quality (Bitew and Alemayehu, 2017; Fowler et al., 2013; Taghipour et al., 2017, 2021).

With mounting demand for more sustainable food production, there has been a significant surge in the exploration of pioneering technologies aimed at achieving sustainable agriculture and mitigating environmental impacts (Zhu and Chen, 2002). As an environmentally friendly agricultural system, organic farming, relying on organic fertilizers from crop residues, livestock manure, and even human excreta, has been suggested (Reganold and Wachter, 2016; Meemken and Qaim, 2018). However, while emphasizing natural pest control and

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minimizing pollution from synthetic substances, the slow release of plant-accessible nutrients, such as nitrogen and phosphorus, from organic fertilizers remains a significant limitation. Studies report that only 35–53% of nitrogen from various manures is released over 6 months (Agehara and Warncke, 2005). Consequently, organic crop production is up to 25% lower than that of conventional agriculture using chemical fertilizers (Forster et al., 2013), highlighting the need for research into expediting the organic fertilizer mineralization process (Wu et al., 2019). Furthermore, in conventional farmland, the availability of oxygen (essential for enhancing metabolism as well as microbial activity) in the soil primarily relies on air diffusion, which is often restricted, particularly in the deeper layers. Therefore, an effective approach for delivering ample oxygen into the soil is yet another desideratum, *inter alia*, in seeking out more sustainable oxygen-delivery strategies for crops, as part of the wider "sustainability" agenda in agriculture.

As part of the urgency in addressing food-sustainability and -security concerns and optimizing crop productivity, nanobubbles (NBs) have emerged as one prominent subject of research within agricultural innovations, offering the promise of sustainable crop production (Wang et al., 2021a), and the present study seeks to gauge the promise of these in crop production, although many open questions remain as to their effectiveness, as we shall outline below. Nanobubbles are acknowledged as submicron entities with dimensions typically ranging from tens to hundreds of nanometers in diameter (Parker et al., 1994), and manifest remarkable physicochemical properties. Notably, they boast high gas retention capabilities, an elongated lifespan, considerable surface area, and present prominently negative zeta potentials (Bunkin et al., 2016; Jannesari et al., 2018; Liu et al., 2013). These exceptional attributes, coupled with their environmentally friendly nature (as more recent NB-generation methods do not involve the use of chemicals (English, 2022)), have rendered NBs a subject of interest across various disciplines. Specifically, they have attracted attention in the realms of biomedicine (for applications such as drug delivery (Batchelor et al., 2021) and antibacterial treatments (Jannesari et al., 2020, 2023a)), environmental science (particularly in the domains of wastewater treatment and remediation (Levitsky et al., 2022; Atkinson et al., 2019; Taghipour and Ayati, 2015)), and agriculture (Wang et al., 2021a, 2021b; Xue et al., 2023), where they hold the potential for promoting enhanced plant growth in a "green" manner, without leaving any contamination.

In recent studies, the catalytic effects of oxygen, nitrogen, and carbon dioxide nanobubbles on plant growth have been extensively documented (Atkinson et al., 2019). Oxygen nanobubbles have been particularly noted for their ability to enhance physiological processes near plant roots, thereby promoting overall plant growth (Agehara and Warncke, 2005; Wang et al., 2021b; Ahmed et al., 2018).

While significant progress has been made in leveraging nanobubbles for crop cultivation, there is a need to explore the potential advantages of utilizing air nanobubbles as alternatives to specialized gas variants, driven by economic and accessibility factors. However, it is worth noting that, heretofore, no significant constructive effects were observed with the use of air nanobubbles (Ahmed et al., 2018) – or, at least, those generated by classical, mechanical methods. Furthermore, while nanobubbles show significant promise in agriculture, particularly in improving nutrient delivery due to the assumption that a considerable portion of nutrients adheres to their surfaces, a notable gap persists in determining the optimal levels of nutrients/fertilizers necessary. This study aims to address these limitations by investigating the novel use of air nanobubbles to minimize fertilizer consumption while simultaneously enhancing crop yield. This innovative approach not only advances sustainable agricultural practices but also provides a practical solution for optimizing nutrient management in crop cultivation. In addition, the utilization of simple and environmentally benign techniques for the large-scale production of pristine bulk nanobubbles, without the need for high energy, additives, chemicals, compressed air/oxygen, oxygen manufacture or, indeed, moving parts (subject to inevitable breakdown), serve to enhance the potential scope of nanobubble applications involving radical and technically/commercially scalable new NB-generation methods.

In the present study, we seek to apply a promising new NB-generation method to avoid the need to resort to less scalable and more expensive NB approaches (particularly in terms of operating – e.g., energy and maintenance – costs), as well as avoiding the use of chemical agents or compressed gas or oxygen manufacture (e.g., pressure-swing adsorption). This is with a view to assessing both germination and plant-growth characteristics of such a new and highly scalable method. In particular, we assess a novel, low-energy, and additive-free method of generating large populations of NBs based on applying an external electric field – without the need for the NB generator to have moving parts – which is also suited and scalable for open-field agriculture (English, 2022). To unravel the intricate mechanisms driving growth in the presence of air NBs, we conducted a pioneering investigation into the generation of reactive oxygen species (ROS) throughout the germination process. This groundbreaking study sheds light on the potential role of ROS in enhancing seed vigor and promoting early growth stages when exposed to electric-field-generated air NBs. This leads, naturally, to the use of air NBs in the present work to stimulate both seed germination and plant growth using reduced levels of fertilizer (owing to fertilizer adsorption on NB "carriers" (English, 2022)) presents considerable potential in curbing reliance on traditional fertilizers and reducing the risks associated with environmental contamination (Taghipour et al., 2022).

2. Materials and methods

2.1. Nanobubble generation

Air nanobubbles were generated in very large quantities (and density per unit volume) via a submerged NB generator through an electric-field approach (Ghaani et al., 2020), provided by AquaB Nanobubble Innovations Ltd. (Ireland, www.aqua-bubble.com). The NB-generation process was triggered by applying a DC voltage of 300 V (with sub-milliAmp current levels, ~0.1–0.15 mA), while air was injected into the 1.5 L water system (open to atmosphere) by a diffuser (Hygger S100) in municipal Dublin tap-water for 30 min. The meso-bubbles generated by the diffuser rose through the DC electric–field milieu inside the NB generator, creating a sub-population of NBs, which diffuse throughout the body of water.

2.2. Characterization of water in the absence and presence of NBs

The electrical conductivity (EC), pH, oxidation-reduction potential (ORP), and dissolved oxygen (DO) of the water samples were measured before treating the plants by utilization of a portable multi-parameter instrument (WTW multi 3630 IDS, Germany). The size distribution and concentration of the produced NBs in tap water were measured via a Zetasizer Pro (Malvern Instrument Ltd, UK) based on the Stoke-Einstein equation. These parameters were measured through the Dynamic-Light Scattering approach, with the source light of a 633 nm laser. In addition, the zeta potential of tap water in the absence and presence of NBs was examined using the same instrument. All the measurements were carried out at a constant temperature (room temperature $\sim 25 \pm 1$ °C) through a temperature controller, installed on the system.

2.3. Germination-rate investigation

The lettuce and basil seeds utilized in this study were procured from a local market in March 2023. Detailed information about these seeds can be found on the packaging in supporting information (Fig. S1). The germination rates of both treated (with air NBs) and untreated lettuce and basil seeds were determined daily by calculating the ratio of germinated to the total number of seeds in each group as the following equation:

$$\text{The germination rate} = S_g/S_t \quad (1)$$

where S_g is the germinated seeds and S_t is the total number of seeds.

In this experiment, each group consisted of 60 seeds in which 30 seeds from each group were placed in a separate 500 mL container. These containers were filled with either regular tap water or tap water infused with air NBs. All the samples were stored at dark conditions and room temperature (~22 °C). As part of the essential maintenance routine during germination, aimed at preventing water evaporation and oxygen depletion, both the tap water and air nanobubble (NB) solutions were renewed every 24 h. For a more comprehensive exploration, we extended our investigation to include the measurement of germinated seed size at the germination rate course. Measurements of hypocotyl lengths were conducted daily to facilitate a comparative analysis of hypocotyl elongation by capturing images of all tested seeds each day. These images were then subjected to analysis using the ImageJ software, enabling quantifying the hypocotyl length of the seeds accurately. To better demonstrate the germination rate, the magnified pictures of NB-treated and untreated seeds were captured through a USB Digital Microscope Camera with an objective lens of 20X-200X portable microscopy.

2.4. Plant-growth study

To delve into the impact of electric field-generated NBs on plant growth, we initiated a study wherein lettuce seeds were cultivated within soil-filled round planters (with a diameter of ~7 cm). Two seeds in each group were cultivated in one planter. The seeds were categorized into two distinct groups: untreated samples, which were irrigated using standard tap water, and NB-treated group. To comprehensively explore the potential effects of the NBs in conjunction with varying fertilizer concentrations on the growth of the lettuce plants, the samples were then treated with different levels of fertilizer treatment (Nutri One Concentrated Fertiliser for All Plant Types with an NPK ratio of 5.5:5:7.5). Accordingly, both the control and NB-treated groups were exposed to a range of fertilizer concentrations after two weeks of their planting when their third shoots appeared. For the control groups, the concentrations were set at 0 and 100%. In contrast, the NB-treated groups experienced a broader spectrum of concentrations, encompassing 0, 25, 50, and 100%. The groups were irrigated by tap water and air-NB solutions approximately every 2–3 days (at the same time). Ultimately, the results were reported as average ± standard deviation.

2.5. Reactive oxygen species generation study

The formation of reactive oxygen species (ROS), specifically hydroxyl radicals ($^{\bullet}OH$) during seed germination was investigated using a specific molecular probe of terephthalic acid (TPA). When exposed to $^{\bullet}OH$ radicals, TPA undergoes hydroxylation, leading to a strong photoluminescence emission at approximately 425 nm. This emission served as an indicator of the presence of hydroxyl-type ROS (Jannesari et al., 2023a). To study this investigation, TPA was administered into the germination media in both control samples (prepared without seeds) and test samples (with lettuce and basil seeds), using fresh air NBs solution or regular tap water. The experiments were conducted under conditions identical to those applied in the germination tests. After 10 and 24 h, the 3 ml of both the control and sample group solutions (homogenized through shaking) were pipetted for PL measurements, and their respective intensity was recorded using a Cary Eclipse Fluorescence spectrometer.

2.6. Statistical analysis

Statistical analysis of the data was conducted by utilizing SPSS software (Version 26; SPSS, Inc., Chicago, IL, USA). This analysis involved performing a one-way analysis of variance (ANOVA) while considering confidence levels of 99% and 95% for statistical significance ($p < 0.01$ and $p < 0.05$, respectively). The results are reported as the mean ± standard deviation of a minimum of three tests.

3. Results and discussion

3.1. Water characterization in the absence and presence of air NBs

The size distribution of the electric field-based air nanobubbles (EF-ANBs) in tap water with a concentration of 3.45×10^9 mL^{-1}, depicted in Fig. S2A demonstrates a very sharp peak at around 52.9 nm. As can be seen from Fig. S2B, the pH of both air NB and the tap water samples was maintained in the range of neutral (between 7 and 8). Fascinatingly, as shown in Fig. S2B, air NB solutions demonstrated a slightly lower pH all over the entire experimental duration (~3 months). This observation can be attributed to the higher air content in the NB water (well above saturation, even excluding the additional mass in the NB form) - including additional CO_2, resulting in the formation of carbonic acid upon dissolution in water (Mohammadian et al., 2023). On the other side, the dissolved oxygen (DO) level in the air-NB water consistently exhibited higher levels during the test period compared to that in the tap water. In fact, the introduction of air-NBs into the liquid resulted in a significant increase in gas uptake, surpassing the predictions of Henry's Law. Moreover, it constantly exceeded 100% of the maximum theoretical dissolved oxygen content at the respective temperature, as illustrated in Fig. S2C. This outcome aligns with expectations, as the air-NB water was anticipated to have a higher DO content than tap water, maintaining (super-) saturation for longer, owing to the DO probe being unable to measure, ipso facto, the level of additional oxygen dissolved in the "nano-phase", and Fick's-Law concentration gradient from the additional "nano-dissolved" oxygen in maintaining the ostensible, conventional level of DO. The oxidation-reduction potential (ORP) exhibited fluctuations in both water samples (Figure S2D). In the case of tap water, the ORP consistently ranged between 200 and 290 mV. Conversely, the ORP of air NB water demonstrated a higher range, fluctuating between 220 and 320 mV. This discrepancy distinctly suggests that the redox potential of air-NB water tended to exceed that of tap water, signifying an elevated oxidation capacity in the water containing air NBs (Kim et al., 2000). Indeed, this serves as a rather convenient "proxy" measure, for convenient (open-)field use of enhanced oxidative capacity afforded by air-NBs – although this is, of course, not as sophisticated as direct methods to probe additional oxygen and NB presence more directly, e.g., light-scattering.

3.2. Effect of air NBs on germination rate and hypocotyl length of the seeds

Fig. 1 illustrates the germination rates and the hypocotyl growth length of lettuce and basil seeds that were subjected to two different treatments of regular tap water and an air-NB solution. As depicted in Figs. 1 and 2, the NB solution prompted a rapid germination rate for both lettuce and basil seeds from the initial day of immersion. In contrast, the untreated seeds only began germinating after the second day, and their rate of germination was notably slower when compared to the treated seeds.

Remarkably, following a 2-day submersion in the EF-ANB solution, the germination rate witnessed a substantial increase, elevating from 20% to 96% for lettuce and from 16% to 53% for basil, respectively. As demonstrated in Figs. 1 and 3, the utilization of air nanobubbles (NBs) has notably accelerated the elongation of hypocotyls in both lettuce and basil seeds. A comparative analysis with conventional tap-water

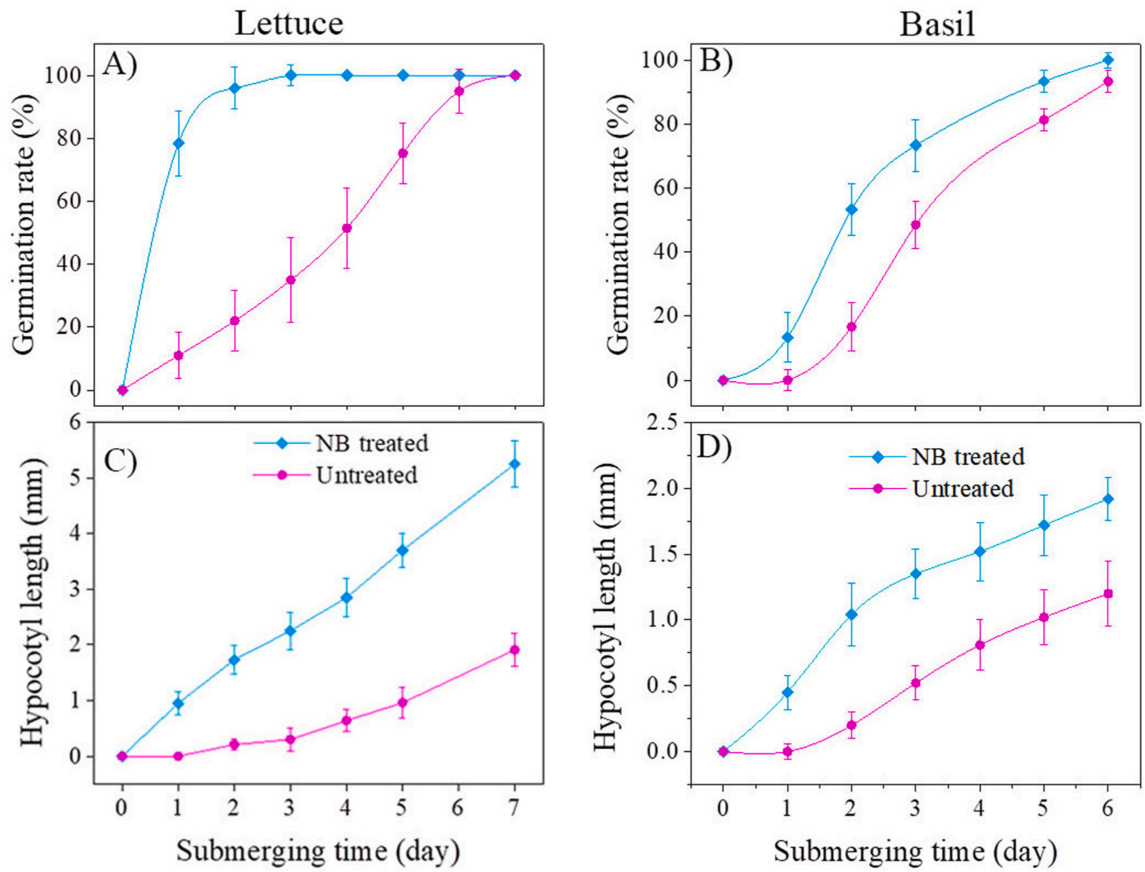

Fig. 1. The germination rates (A and B) and hypocotyl growth length (C and D) of lettuce and basil seeds, treated with air NBs and regular tap water, respectively. The germination rate is calculated as the number of germinated to the total amount of the seeds.

treatments highlights the significant impact of air NBs on the enhancement of basil seed hypocotyl elongation, surpassing the growth observed following immersion in standard tap water over an equivalent temporal span. This augmentation becomes particularly apparent upon a mere 6-day submersion period, where the basil seeds subjected to air NB treatment display a hypocotyl length equivalent to that of seeds immersed in tap water for a substantially protracted duration (10 days). This effect was even more pronounced in the case of lettuce seeds, where an equivalent hypocotyl length was attained within a notably shortened timeframe. It means that after a submersion period of 2 days, the lettuce seeds treated with air nanobubbles (NBs) exhibited hypocotyl lengths equivalent to those achieved after 7 days of submersion with regular tap water. This observation emphasizes the accelerated growth response generated by air NBs compared to the conventional tap water approach.

3.3. *Effect of air nanobubbles and fertilizer treatments on lettuce plant growth*

In the pursuit of enhancing plant growth and productivity, various parameters are commonly employed to assess the development of plants. This study focuses on assessing the growth of lettuce plants by evaluating crucial parameters such as stem, shoot, and root lengths, along with shoot count. Additionally, the research investigates the impact of ANB in conjunction with a single treatment, but different concentrations of fertilizer on lettuce growth (Fig. 4). The experimental design involved distinct treatment groups, including lettuce treated with regular tap water with or without 100% fertilizer, and lettuce exposed to meticulously prepared ANB treatments with different percentages (100%, 50%, 25%, and 0%) of fertilizer. It is worth mentioning that the single fertilizer treatment was applied on the 7^{th} day of the experiment when the third shoots of the plants appeared. The growth progress was tracked over the course of several days following planting.

Results indicated in Fig. 5 demonstrated that in contrast to the reported literature (Ahmed et al., 2018), irrigating with only ANBs dramatically induces an increasing effect not only on the length of stems, shoots, and roots but also on the leaf number of the lettuce plants. In fact, treating the lettuce with just air NB solution accelerated the growth rate of these plants (see Fig. 5).

On the other side, analyzing the data highlights the interplay between air NBs, fertilizer concentrations, and lettuce growth. The outcomes suggest a nuanced correlation, where specific combinations of air NBs and fertilizer percentages distinctly influence the growth patterns of lettuce plants. Remarkably, lettuce plants showcased diverse responses to varying fertilizer levels when irrigated with regular tap water or water enriched with air NBs.

As shown in Fig. 5A, it is evident that the stem length of lettuce plants, irrigated with regular tap water increased by 35 % after the application of 100% fertilizer, and 58 % after enrichment with solely air NBs, excluding any fertilizer. Further significant enhancements of 100 % and 82 % were recorded when air NB solution contained 50 and 100 % fertilizer, respectively. Similar trends were obtained for the length of the shoot and root lengths of lettuce plants over a 30-day experimental period, further corroborating the growth-promoting influence of enriching tap water with air NBs, followed by a combination with fertilizer. This can be attributed to the unique properties of NBs, including their remarkable capacity to impact both active and passive diffusion pathways within plant cells, along with regulating transport proteins. The significant surface charge enables NBs to establish an electric double layer with nearby nutrient cations, thereby helping to generate a localized concentration gradient – serving to transport gases, micronutrients, and bioactive compounds directly to plant roots and foliage, enhancing nutrient absorption. This process facilitates improved

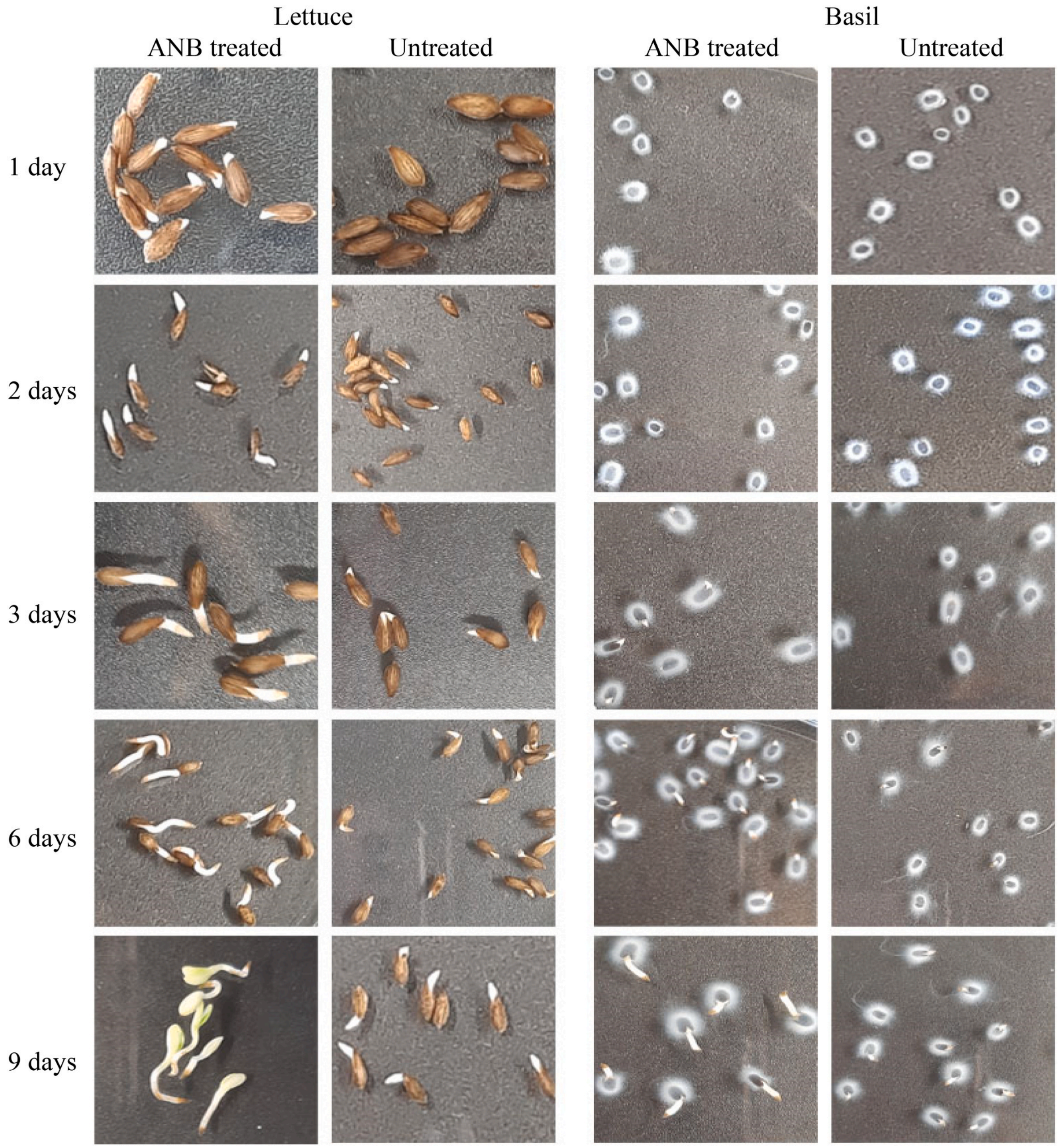

Fig. 2. Digital photos of submersed lettuce and basil seeds in either electric field-based air nanobubbles (EF-ANB (solution or tap water at various time points, depicting the germination rates (as the ratio of germinated seeds to the total number of seeds).

nutrient absorption across cortex cells in root tissue and assists in the release of hydrogen ions from the root zone, crucial for maintaining the plant's ionic balance. This efficient mechanism of nutrient delivery can significantly support plant growth and development (Wang et al., 2021a; Ahmed et al., 2018; Marcelino et al., 2023).

Moreover, lettuce plants treated with ANB solutions containing 100%, 50%, and 25% fertilizer treatments exhibited a remarkable surge in the number of leaves, reaching a maximum of 6 leaves within the observation period, respectively. Among the various treatments, the highest frequency of 5 leaves was observed in the plants treated with air NBs featuring 50% fertilizer. This was closely followed by plants treated with ANBs without and then those containing 25% and 100% fertilizer. In contrast, the treatment involving tap water with 100% fertilizer exhibited a comparatively lower leaf distribution.

Overall, the dynamics shifted when tap water was replaced with NB-enriched water. In this scenario, the application of air NBs surpassed the growth-promoting effect of fertilizer in regular water. The lettuce plants exhibited non-linear, concentration-dependent responses when exposed to the combined treatment of NBs and fertilizer. Nanobubbles (NBs) play a pivotal role in enhancing plant water uptake. When introduced into irrigation water, NBs elevate their oxygen content (as depicted in Fig. S2C), thereby promoting improved root health and function. This enhancement facilitates more efficient water absorption by plants, especially crucial during key growth phases, ultimately fostering robust and vigorous plant growth. Furthermore, the oxygen-rich environment created by NBs encourages aerobic respiration, leading to increased metabolic activity. This metabolic boost enables plants to utilize macro and micronutrients more effectively, further supporting overall plant growth and development (Wang et al., 2021a).

Intriguingly, applying 25% fertilizer to NB-enriched water did not yield any notable changes in plant growth. However, the use of 50% fertilizer in conjunction with air NB-enriched water resulted in even

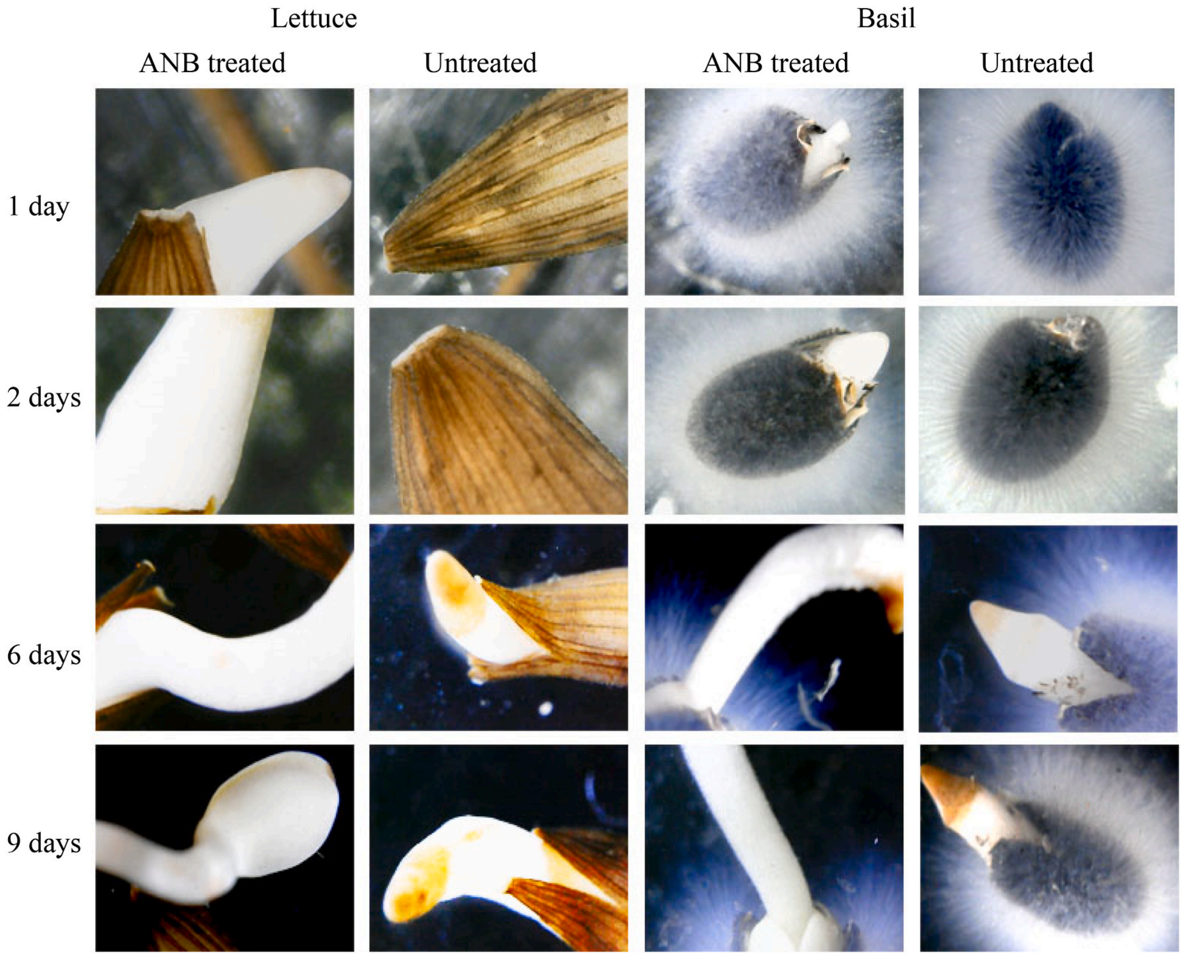

Fig. 3. Microscopic photos of the hypocotyl growth of lettuce and basil seeds submersed in EF-ANB water and tap water at different time courses.

more substantial growth enhancement compared to the use of 100% fertilizer. Therefore, this strategy, requiring no additional special gases, offers eco-friendly alternatives for optimizing plant nutrition and accelerating growth, potentially increasing crop yields.

3.4. Mechanism study

During seed germination, an increase in metabolic activity, particularly in the mitochondria enhances the respiration process, leading to the production of ROS as byproducts (Gomes and Garcia, 2013; Fang et al., 2023). These ROS can serve as signaling molecules to activate specific enzymes needed for germination (Bailly, 2019). Interestingly, seed germination appears only when the level of ROS within the seed reaches an adequate threshold to enable ROS signaling and trigger a transition from a dormant to a non-dormant state. However, germination is inhibited when the ROS concentration is either too low (as seen in dormant seeds) or excessively high (common in aged seeds or seeds exposed to unfavorable germination conditions) (El-Maarouf-Bouteau and Bailly, 2008).

Investigating the impact of NBs on seed germination, we introduced terephthalic acid (TPA) as a specific molecular probe for detecting hydroxyl radicals ($^{\bullet}OH$) (Page et al., 2010). TPA was added to the germination media of both regular tap water and air NB water, in the absence and presence of lettuce and basil seeds under conditions mirroring the germination tests. The results in Fig. 6 revealed that basil seeds, followed by lettuce seeds in air NB water, exhibited the most pronounced photoluminescence emission associated with reactive oxygen species generation. Notably, air NB water alone, in the absence of seeds, also displayed ROS generation, albeit with a substantial difference. In contrast, for regular tap water, no significant variations were observed in ROS generation when comparing the presence and absence of lettuce and basil seed models. Analyzing the data reveals that ROS stimulation for both models of the seeds was dramatically boosted when they were exposed to air NBs.

Mitochondria, often referred to as the cellular "energy factories" within plant cells, play a pivotal role in generating energy through the process of respiration (Wang et al., 2022). During respiration, complex redox reactions break down organic molecules like glucose to produce energy. These complexes are responsible for passing electrons along a chain of redox reactions, creating a flow of electrons (Auger et al., 2021). In these reactions, electrons are transferred from a donor to an electron acceptor with a higher electronegative affinity called an electron transport chain (ETC). In aerobic respiration, molecular oxygen serves as the ultimate electron acceptor, combining with proton ions to generate water molecules and release energy (Foyer and Harbinson, 2019). During this process, there is a possibility of electron leakage from the ETC (Jannesari et al., 2024). If a potentially leaked electron encounters an oxygen molecule before completing the respiration chain, the creation of reactive oxygen species becomes inevitable (Alber et al., 2017). Therefore, increasing the DO level due to the NB formation introduces more oxygen into the system, resulting in more ROS stimulation.

Extending the duration from 10 to 24 h, the amount of ROS stimulation in both basil and lettuce seeds submerged in regular tap water was notably increased compared to the control group (tap water alone). It is worth noting that the ROS levels in these seeds remained significantly lower than those observed in the seeds exposed to ANBs. This phenomenon can be ascribed to the increased leakage of electrons directed

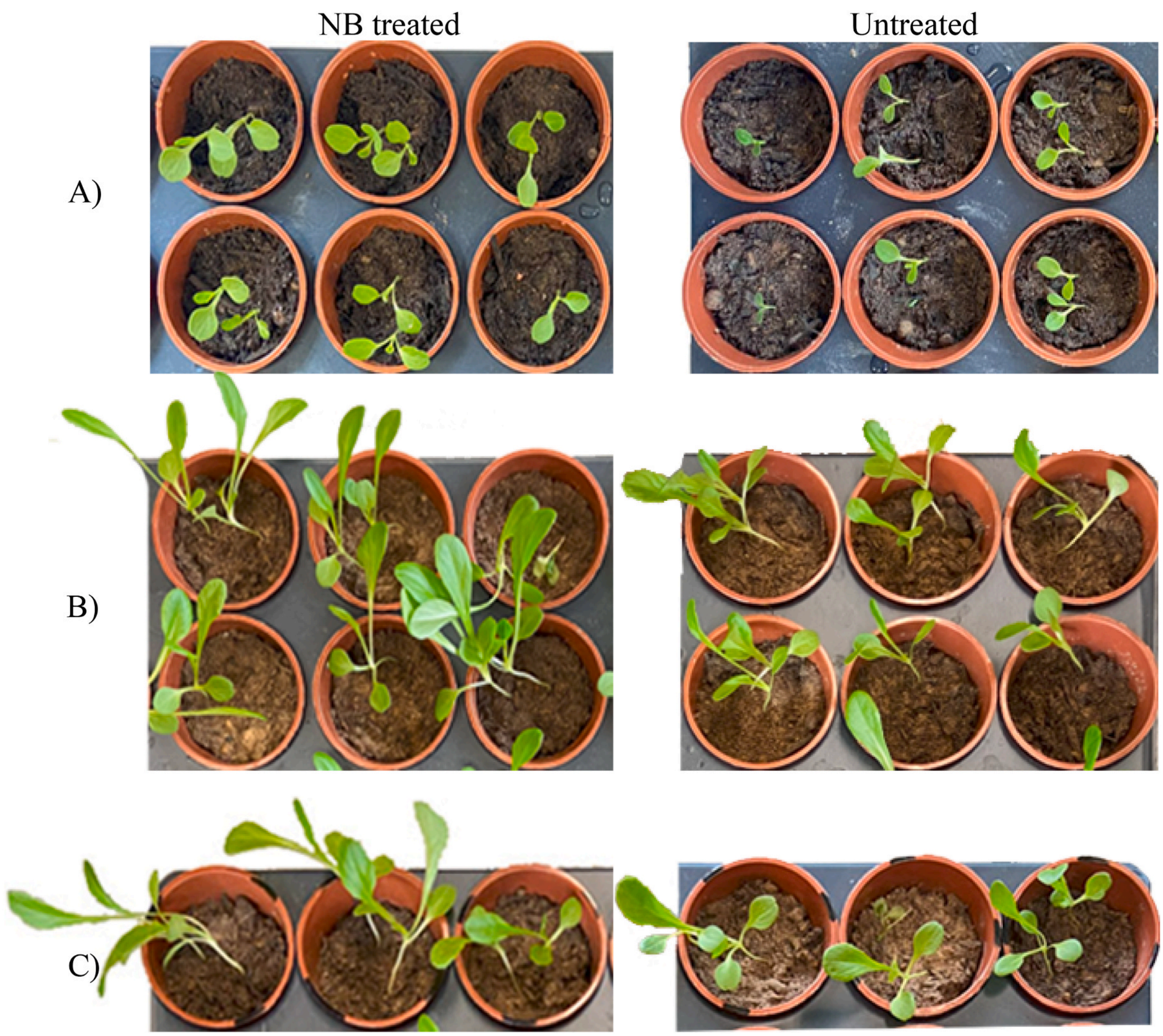

Fig. 4. Digital photos of different stages of lettuce plants irrigated with the same amount and frequency of either EF-ANBs or regular tap water (A) right before fertilizer treatment (on the 7th day) and after receiving (B) following a single fertilizer application (at the 30th day of planting), and (C) no fertilizer treatment.

toward the accessible oxygen during the respiration process over an extended period (Jannesari et al., 2023b). Furthermore, variations in ROS production were observed between the basil and lettuce seeds as they grew.

It can be attributed to the micellar form of the basil seeds in the water (Fig. 3), enabling them to trap more TPA accessible to OH radicals around the seeds.

3.5. Sustainable agriculture: cutting fertilizer consumption and energy expenses

In 2023, global fertilizer consumption surged to 191.5 million tons, with nitrogen (N) accounting for approximately 57% of the total consumption and phosphate (P_2O_5) comprising 24%. The increasing global demand for NPK fertilizers, projected to reach 324 million metric tons by 2050, underscores the significance of fertilizer consumption (I.F.A.P.S. M.-T.F.O. IFA).

Although the widespread adoption of synthetic fertilizers has substantially increased agricultural productivity, enabling farmers to cultivate more crops with fewer land resources, the rise in fertilizer usage has led to significant drawbacks, particularly in the form of greenhouse gas emissions. After fossil fuels, agriculture now ranks as the second-largest contributor to global climate change pollution (Lynch et al., 2021). The manufacturing and application of fertilizers contribute significantly to this, with nitrogen fertilizer alone accounting for 47% of agricultural emissions (Franks and Hadingham, 2012). Decreasing NPK fertilizer consumption through applying NB technology holds promise in mitigating environmental damage by reducing the likelihood of greenhouse gas emission and nutrient runoff into water bodies, thereby combating eutrophication and water pollution, and ultimately preserving water quality and ecosystem health (Zhang et al., 2021). On the other hand, soil deterioration quality by overusing fertilizer can be alleviated, promoting healthier soils and sustainable agricultural methods (Lenka et al., 2016; Massah and Azadegan, 2016). It is noteworthy that the reduction in fertilizer usage not only leads to cost savings for farmers but also enhances overall profitability.

To assess the energy-economics advantages of replacing, partially (of the order of three quarters, as the present study suggests) synthesized-fertilizer (NPK) levels with EF-ANB technology, we can begin, in a "life-cycle" sense, by estimating the energy consumption involved in fertilizer production. Based on calculated energy consumption rates of 5.7 and 1.86 GJ/t (Gigajoule per Metric Tonne), for nitrogen (N) and potassium (K) respectively, and considering annual worldwide production quantities of 109.1 million tonnes of N and 46 million tonnes of K, total annual energy consumption is estimated at 622.1×10^6 GJ for N and 85.5×10^6 GJ for K (Daramola and Hatzell, 2023). Assuming that the EF-ANB method replaces roughly half of the fertilizer level for 1% penetration of global crop farming, this would amount to an annual

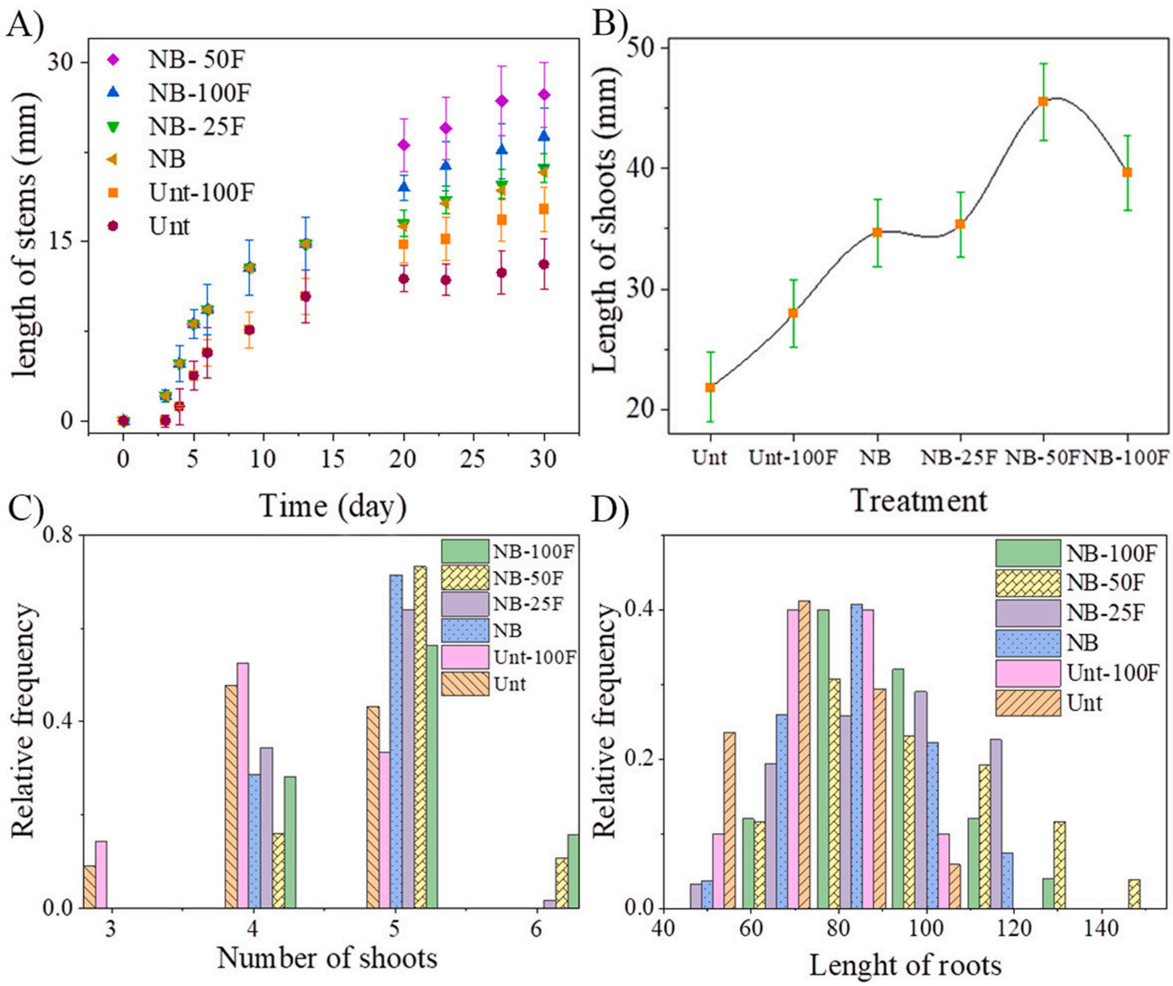

Fig. 5. The impact of combined treatment of air NBs with a single-time fertilizer application at various concentrations (0, 25, 50, and 100%, corresponding to NB, NB-25F, NB-50F, and NB-100F) on plant growth in comparison to the effect of regular tap water in the absence and presence of 100% fertilizer, indicated as Unt and Unt-100F, respectively.

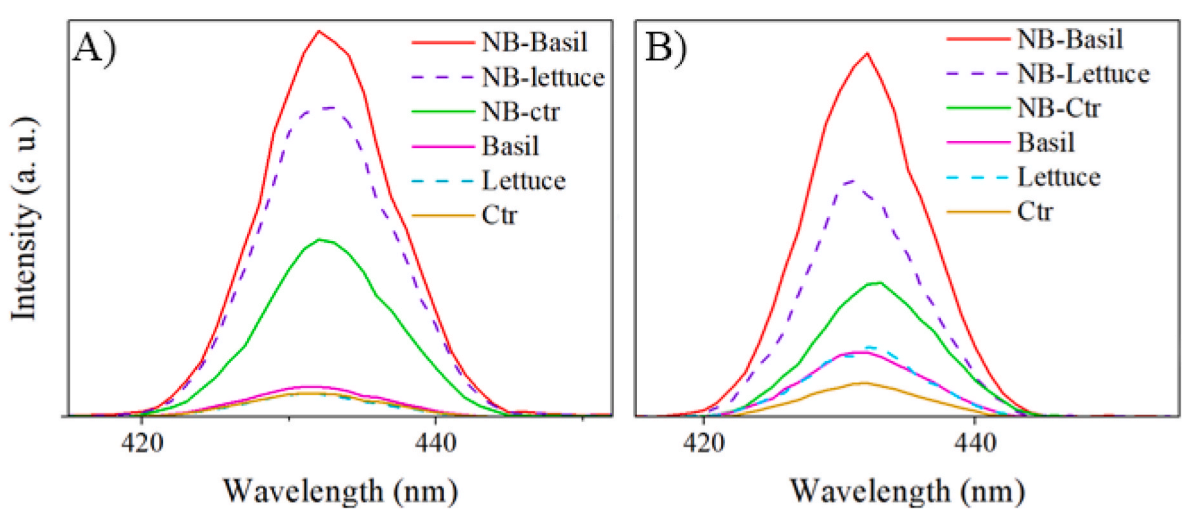

Fig. 6. The photoluminescence intensity of Terephthalic acid (TPA) exposed to lettuce and basil seeds submersed in air NB and regular tap water after (A) 10 and (B) 24 h, relatively indicating ROS (specifically OH radical) generation.

energy saving of 3.53×10^6 GJ.

Conversely, when considering the energy consumption for each operation of the NB generator to produce NBs through the electric field strategy, the formula is as follows:

$$\text{Energy consumption} = N \times V \times I \times t$$

Here, N denotes the number of generators (of the submersible type referred to in the present study), V represents voltage (300 V in an AquaB prototype unit), I denotes current ($\sim 0.1 \times 10^{-3}$ A in an AquaB prototype unit), and t stands for the duration of each operation (1 year, or 31.54×10^{6} s). For an estimated 100,000 installed units for 1% of crop-market penetration, the level of annual energy. Consequently, the energy consumed per operation is calculated as 94.62 GJ – which is utterly trivial compared to 3.53×10^{6} GJ in terms of "displaced/offset fertilizer" production-energy costs. Even discounting entirely, the use of solar energy, and using of the order of $0.1 per kWh as the price of agricultural mains (AC) electricity and allowing several-fold inefficiency in AC-to-DC conversion, this leads to annual energy savings of ~$140 million – neglecting savings in transport costs for this offset fertilizer.

In terms of environmental implications of scale-up, this can be quantified in the level of fertilizer-usage reduction – and the resultant reductions in groundwater pollution by fertilizer run-off in water sources (Lynch et al., 2021; Franks and Hadingham, 2012; Zhang et al., 2021; Lenka et al., 2016; Massah and Azadegan, 2016; Daramola and Hatzell, 2023), and other environmental fates of the fertilizers from field run-off and groundwater drainage (Swaney and Howarth, 2019; Sun et al., 2012; Bitew and Alemayehu, 2017). Fully one-third of global methane emissions arise from under-aerated waterways undergoing eutrophication – chiefly from fertilizer contamination (Beaulieu et al., 2019). Therefore, the reduction in fertilizer usage afforded by EF-ANB deployment will result in a suppression of eutrophication pressures on water bodies. In addition, the application of solar-powered submersible NB generators for water-body aeration also assists in maintaining dissolved-oxygen levels therein, even if there is undesirable fertilizer-runoff occurring thereto (itself mitigated by reduced fertilizer usage in agriculture).

4. Conclusion

This study demonstrates the potential of electric-field-generated air nanobubbles (EF-ANBs) in irrigation to enhance agricultural crop efficiency sustainably. This innovative approach reduces reliance on fertilizers, thereby promoting eco-friendly practices. Notably, EF-ANBs require minimal energy, involve no moving parts, and entail low maintenance.[19] The effect of EF-ANBs on overall plant growth was explored in the present study based on a submersible Aqua-B generator (which may be retro-fitted easily to irrigation header tanks, and, of course, solar powered - although in-line models are naturally available for incorporation into irrigation-piping networks, not considered in the present study). The results revealed a substantial improvement in both germination rates and plant growth when EF-ANBs were applied. Immersion in air nanobubbles resulted in a notable increase in germination rates, with lettuce and basil showing approximately 5 and 3.5 times higher rates, respectively, compared to the control group. Moreover, EF-ANB treatment led to accelerated hypocotyl elongation in both lettuce and basil, reducing the duration from 7 to 2 days and from 10 to 6 days, respectively. These improvements can be attributed to the increased induction of reactive oxygen species (ROS) facilitated by EF-ANBs. By providing elevated oxygen levels, air NBs enabled a concurrent rise in aerobic respiration and greater electron leakage from the ETC, leading to heightened ROS generation - signaling the stimulation of growth. Furthermore, the application of EF-ANBs in irrigation outperformed the effects of conventional fertilizers (owing to more efficient delivery of adsorbed fertilizers to NBs, as carrier agents, and passage through the plant-cell-wall matrix),[19] demonstrating a strong catalytic effect on the growth of shoot, stem, and root length, as well as the leaf count of lettuce plants. Plants irrigated with a single application of fertilizer alongside EF-ANBs exhibited superior growth compared to plants subjected to regular tap water, including various concentrations of conventional fertilizers and those left untreated. The utilization of EF-ANBs irrigation significantly augmented the growth of plants subjected to a single application of fertilizer, as compared to plants treated with fertilizer in conventional tap water. These findings highlight the synergistic interplay between aerobic respiration and ROS generation in promoting plant growth, particularly when fertilizer levels are reduced due to the presence of EF-ANBs. This promising correlation shall no doubt pave the way for introducing a more sustainable solution for eco-friendly agriculture in the future, owing to the inherent simplicity and scalability of the platform electric-field NB-generation technology – *inter alia*, no moving parts, low energy, solar power for off-grid work on land and water, no chemicals or additives.[19] The prospect of rendering water usage more productive in agriculture, as well as crop growth per se, in tandem with less fertilizer in run-off water (and associated cyanobacteria and water-quality problems in water bodies) is a fine example of NB sustainability galvanizing the whole farming-water life-cycle for greater sustainability.

CRediT authorship contribution statement

Marziyeh Jannesari: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Anna Caslin:** Writing – original draft, Methodology, Formal analysis. **Niall J. English:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.121228.

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