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Micro-nano bubble aeration promotes senescence of submerged macrophytes with low total antioxidant capacity in urban landscape water

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Macrophytes and micro-nano bubbles (MNBs) have increasingly been used for restoring natural waters suffering from eutrophication. However, the effect of MNBs on macrophytes has not drawn great attention. This study provides insights into how MNBs affect macrophytes by analyzing the growth indicators of three species of macrophytes, total antioxidant capacity (T-AOC), malondialdehyde (MDA), superoxide dismutase (SOD), proline (PRO) and osmotic potential. Our results suggest that the MNB concentration and aeration frequency should be taken into account in the area of urban water with dense macrophytes.

ARTICLE

Micro-nano bubble aeration promotes senescence of submerged macrophytes with low total antioxidant capacity in urban landscape water

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Rapid industrialization and urbanization have contributed to eutrophication of urban landscape waters in China. As an important part of aquatic ecosystem, macrophytes have many advantages in controlling eutrophication and blooming. In recent years, micro-nanobubbles aeration (MNBs) has increasingly been used for restoring natural waters suffering from eutrophication, which may affect the growth of macrophytes and thereby affect ecological restoration, but the effect of MNBs on macrophytes has not drawn great attention. In this presentation, three common species of macrophyte (*Echinodorus amazonicus* or EA, *Echinodorus quadricostatus* or EQ and *Microsorum pteropus* or MP) were grown for 60 days in water aerated with MNBs and their growth was compared to that of the same plants grown with standard macrobubble aeration. The growth of EA and EQ was significantly inhibited by MNB aeration, and was accompanied by various symptoms of senescence (yellowing and chlorophyll degradation, the inhibition of photosynthesis rate, carbon fixation and plant growth, and the decline of respiration rate). However, the growth of MP plants was not significantly affected under the same conditions. The final fresh weights (FFWs) of EA, EQ and MP in the presence of high MNB levels were 0.75, 0.83 and 1.05 times those in the macrobubble aeration system; we designate this parameter $Ratio_{FFW}$. We found the initial total antioxidant capacity (T-AOC) of EA and EQ to be 48.00 and 64.67 U/mL, respectively, markedly lower than that of MP (617.33 U/mL). The $Ratio_{FFW}$ values and other growth indexes of the three plant species were positively correlated with their T-AOC. Macrophytes use their antioxidant defense mechanisms to prevent oxidative damage by ROS, while still allowing exogenous ROS to function as signalling molecules to regulate plant growth and senescence. Premature senescence is a protective mechanism employed when plants are stressed and their growth is consequently inhibited. Our results suggest that the MNBs may not positively promote the growth dense macrophytes but potentially induce damage on water ecosystem.

1. Introduction

Urban landscape water refers to lakes and rivers in city, which is essential and important to the urban ecosystem and recreational activities. In recent years, rapid industrialization and urbanization have contributed to eutrophication of urban landscape water in China. As an important part of aquatic ecosystem, macrophytes could remove nutrients from water and the allelopathic inhibition effects of macrophytes has many advantages in controlling eutrophication and blooming,[1–3] which play a vital role in restoring the aquatic ecosystem. Therefore, numerous environmentally friendly ecological engineering approaches with macrophytes were used to improve the quality of urban landscape water, such as macrophytes replantation[4], planted floating treatment bed[5] and flocculation-capping that can facilitate the switchover from algae- to the macrophyte-dominated stateand.[6]

Micro-nanobubbles (MNBs) consist of microbubbles (MBs; 10-50 μm) and nanobubbles (NBs; <200 nm) that are characterised by slow rising velocity, long hydraulic retention time, large specific surface area and high transfer efficiency.[7] The surfaces of MNBs are usually negatively charged with zeta potential ranging from about -20 to -17 mV in ultrapure water[8], which stabilizes these bubbles in liquid.[9] A feature of MNBs is self-shrinkage and the shrinkage rate increases with decreasing bubble size.[10] When MNBs shrink and collapse, the rapid increase in the charge density of the electric double layer triggers the generation of many hydroxyl radicals (•OH) and other reactive oxygen species (ROS).[11–13] NBs can remain stable for long periods (up to several months),[14] which were also found to be able to produce ROS continually in water.[15,16] There are two main methods for the generation of MNBs involving either pressurisation or gas-water circulation. The pressurisation method generates smaller bubbles at a higher concentration[14,17,18], which probably results in a

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higher ROS concentration, and there is no evidence that the physicochemical properties of MNBs generated by the two techniques are different.[13]

Micro-nano bubbles (MNBs) have also increasingly been used in urban landscape water treatment due to the higher gas transfer efficiency and other properties, which could facilitate aerobic biodegradation and improve the efficiency of contaminant removal.[19–21] MNBs were used to separate algae from eutrophic water via air flotation[22], which also reduces algae-induced anoxia/hypoxia[23] and controls the nutrient release from sediments[24]. Moreover, MNBs generated by gas-water circulation have been reported to accelerate the growth of lettuce[25,26], spinach[27] and *Brassica campestris*[28]. However, MNBs were also found to inhibit plant (e.g., spinach) growth[17,29] , which has not been well studied, especially on macrophytes in landscape water ecosystem.

In the present study, three common species of macrophyte (*Echinodorus amazonicus* or EA, *Echinodorus quadricostatus* or EQ and *Microsorum pteropus* or MP) were cultivated with the same DO level in water aerated with MNBs and their growth was compared to that of the same plants grown with standard macrobubble aeration. The growth ratio of fresh weight (final fresh weight / initial fresh weight), dry weight, chlorophyll and average length of roots and leaves were measured as indicators of growth; total antioxidant capacity (T-AOC), malondialdehyde (MDA), superoxide dismutase (SOD), proline (PRO) and osmotic potential were measured to analyze the effects of MNBs on macrophytes and its mechanism. The results show that macrophytes have different responses to the effects of MNB aeration and highly concentrated MNBs leads to premature senescence of macrophytes with T-AOC.

2. Materials and Methods

2.1. Preparation and cultivation of plants

Three submerged macrophytes: *Echinodorus amazonicus* (EA), *Echinodorus quadricostatus* (EQ) and *Microsorum pteropus* (MP) were cultivated at 25±1℃ in homogeneous ceramsite sand with an 8 h photoperiod (LED plant lamps, photosynthetic photon flux density 130 μmol·m^{-2}·s^{-1}, AD-ADE, SUNSUN, China). The three plants are easy to cultivate and grow fast with low requirements of fertilizers and carbon dioxide, which allows for rapid observation of their growth characteristics and effects of MNBs and macrobubbles. The plants were cultivated for 15 days before the experiment, then two EA plants, sixteen EQ plants and sixteen MP plants with the same initial fresh weight (weight recorded immediately after the plant was taken from the water and wiped dry) were grown with either microbubble or macrobubble aeration for 60 days. **Fig. 1** shows that plant cultivation was conducted in a polymethyl methacrylate tank with dimensions 80 cm×50 cm×60 cm and a total volume of 240 L. The tank was divided equally into two compartments by a polymethyl methacrylate plate: aeration was carried out using MNBs on the right side and macrobubbles on the left. The eutrophic landscape water was collected from the River (N31°17’7”, E121°91’54”) with an initial DO level at around 7.5 ± 0.34 mg/L mg/L and the total nitrogen and phosphate shown in **Fig. S2**. The

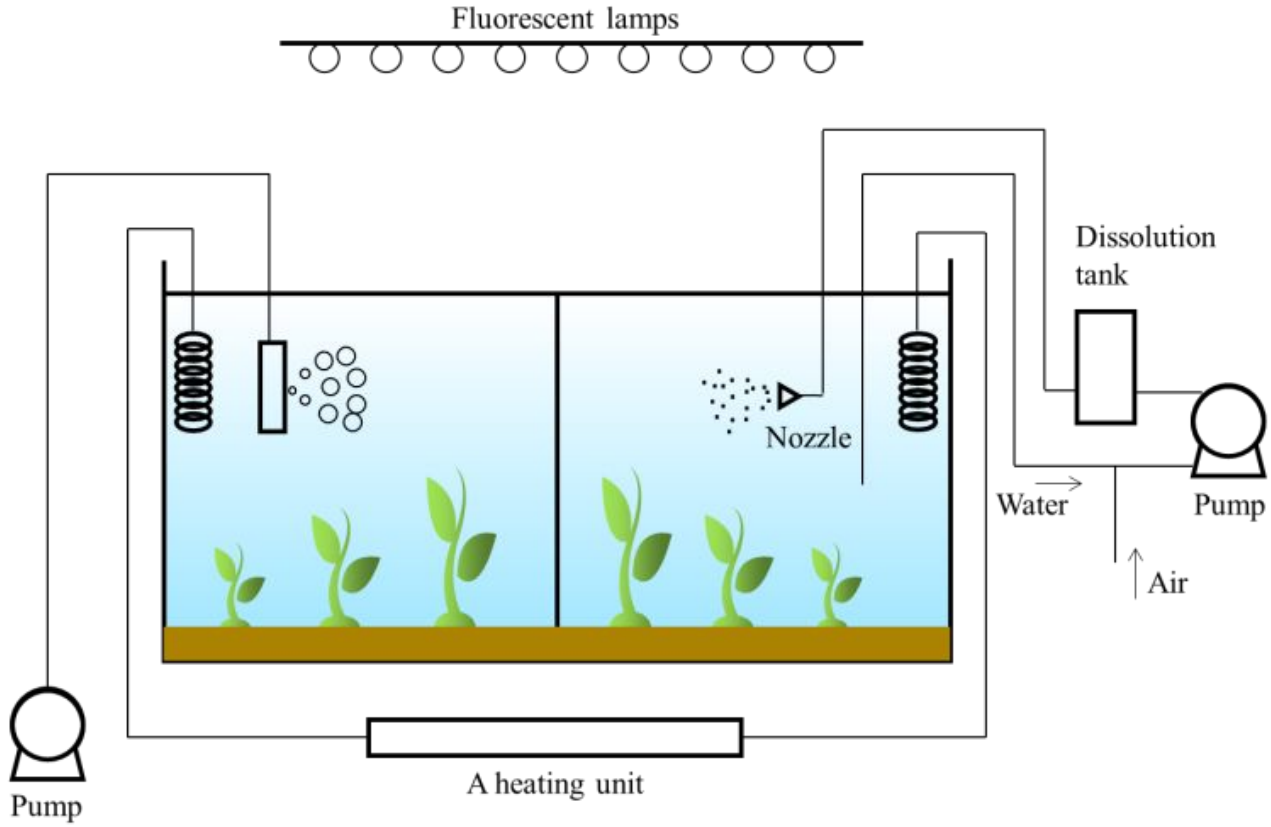

Fig. 1 - The experimental setup for cultivation of submerged plants using two aeration systems (micro-nano bubble, right; macrobubble, left).

DO level was maintained between 8.5 and 9.5 mg/L by adjusting the aeration amounts or frequency of MNBs. After pilot study, the aeration frequency was as follows: MNBs were supplied for for 5~8 min every 30 min, whereas macrobubbles were supplied continuously. The application intensities of MNBs or macrobubbles in our study were chosen to maintain a relatively high level of bubbles and radical concentrations to facilitate the rapid observations of plant growth effect within relatively short periods of time and avoid other potential side effects from plant aging. DO levels in the two compartments were measured by DO meters (HQ30d, flexi, HACH®) every 60 min during the experimental period.

2.2. Preparation of MNBs in water

MNBs were generated using the pressurisation method in our study. The generation system comprised a centrifugal pump, a dissolution tank (300 mL) and an injection nozzle (MF-5000, XINGHENG, China) as illustrated in **Figure 1** (right side). Water and ambient air were introduced into the tank and circulated by the centrifugal pump at flow rates of 11 L/min and 0.14 L/min respectively with a high hydraulic pressure (0.4 MPa). In the control experiment (**Figure 1**, left side), macrobubbles were generated by an air pump (YTZ-312, YEE, China) with an air flow rate of 1.1 L/min. Microbubble size distribution in water was measured at 25℃ by static light scattering using a HORIBA LA-960 instrument (0.01 μm to 5000 μm), while NB size distribution was measured by dynamic light scattering using a Malvern Panalytical NanoSight NS300 instrument (10 nm to 2000 nm) over a 30 min period.

2.3. Evaluation of plant growth with aeration by MNBs or macrobubbles

2.3.1. Measurement of plant growth

After the cultivation period, the fresh weight, root and leaf length of the three plant species on both sides of the apparatus were measured. Dry weight was measured after drying at 80°C for 3 d. The results were expressed as an average ± standard deviation.

2.3.2. Measurement of the indexes in leaves

For each kind of plant, 10 g plant tissue samples were taken randomly from leaves using a hole puncher (diameter = 1 cm); 1 g tissue contains about 20 samples from the hole puncher. The tissue samples were used as described below, with all measurements performed in triplicate.

2.3.2.1. Chlorophyll content

Leaf chlorophyll content was measured by UV-vis spectrophotometry (DR 6000, HACH®). Plant samples were added to 10 ml ethanol (95%), followed by addition of calcium carbonate powder and quartz sand. The mixture was homogenized in a 50-ml glass homogenizer (Manual, Yami, Jiangsu, China) and centrifuged at 3500 rpm for 10 min. The chloroplast extract was measured for absorbance at wavelengths of 665 nm and 645 nm, with 95% ethanol as a blank control. The chlorophyll content is calculated as shown in formulas (1), (2) and (3):

$$\text{Chlorophyll a} = 12.7A_{663} - 2.59A_{645} \quad (1)$$

$$\text{Chlorophyll b} = -4.67A_{663} + 22.9A_{645} \quad (2)$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (3)$$

2.3.2.2. MDA content

The degree of ROS oxidative damage can be estimated by the amount of malondialdehyde (MDA) in leaf tissue, which was measured using a MDA assay kit (TBA method, A003, Nanjing Jiancheng Bioengineering Institute). MDA is a byproduct of lipid peroxidation and can react with thiobarbituric acid (TBA) to produce a red compound, which has a maximum absorption peak at 532 nm. Plant samples were placed in phosphate buffer at a ratio of 1:9 (w/v). The mixture was mechanically homogenized and centrifuged at 3500 rpm for 10 min; the supernatant was then tested according to the manual of the assay kit.

2.3.2.3. SOD content

Superoxide dismutase (SOD) is an enzyme that transforms superoxide radicals into either molecular oxygen or hydrogen peroxide; its activity was measured using a SOD assay kit (WST method, A001-3-2, Nanjing Jiancheng Bioengineering Institute). Plant samples were placed in phosphate buffer at a ratio of 1:9 (w/v) and the mixture was mechanically homogenized and centrifuged at 3500 rpm for 3 min, followed by incubation at 37℃ for 20 min. The SOD concentration (U/ml) was then read from a Synergy™ HT Multi-Mode Microplate Reader at a wavelength of 450 nm.

2.3.2.4. T-AOC content

The total antioxidant capacity (T-AOC) was measured with a T-AOC assay kit (colorimetric method, A015, Nanjing Jiancheng Bioengineering Institute). Plant tissue samples were mechanically homogenized in saline at a mixing ratio of 1:9 (w/v) on ice. The suspension was then centrifuged for 5 min at 12000 rpm at 4℃. The buffer solution, ABT solution, peroxide solution, Trolox solution and samples were then prepared according to the manual of the assay kit, and then the OD value of each tube was read using a Synergy™ HT Multi-Mode Microplate Reader at a wavelength of 405 nm.

2.3.2.5. Relative osmotic pressure

Relative osmotic pressure was measured using a freezing point osmometer (OM819.C, LOSER, Germany). One g plant tissue was frozen at -20℃ for 3 h, then placed in deionized water at a mass ratio of tissue weight (g): volume of deionized water (ml) = 1:9. The mixture was mechanically homogenized and centrifuged at 3500 rpm for 10 min. The supernatant was then used for the calibration, loading, deep freeze and determination steps of osmotic pressure measurement.

2.3.2.6. PRO content

Proline (PRO), which accumulates as a compatible solute against external osmotic stress, was measured with a proline assay kit (acid ninhydrin method, A107, Nanjing Jiancheng Bioengineering Institute). Plant samples were mixed at a ratio

of tissue weight (g): volume of phosphate buffer (0.1 mol/L, pH 7~7.4) (ml) = 1:9 on ice. The mixture was mechanically homogenized and centrifuged at 3500 rpm for 10 min. The supernatant was then tested according to the manual of the assay kit.

2.4. Statistical analyses

All comparisons were made between the control group (macrobubble aeration system) and the test groups (MNB system). One-way analysis of variance was performed followed by Tukey's HSD test with $p < 0.05$ (*) and $p < 0.01$ (**) deemed to be significant.

3. Results and discussion, Experimental

3.1. DO and pH level in water aerated by MNBs and macrobubbles

Fig. 2a shows that during the 60 days of experimental cultivation, the DO levels in the two aeration compartments were almost the same. There was no significant difference in DO level in the nutrient solutions of the two systems, with average DO levels of 8.90 ± 0.32 mg/L (MNBs) and 8.85 ± 0.35 mg/L (macrobubbles). Over a typical 24 h period, the DO levels in the two systems fluctuated between 8 mg/L and 9 mg/L (**Fig. 2b**), with the average DO levels being 8.39 ± 0.12 mg/L (MNBs) and 8.45 ± 0.23 mg/L (macrobubbles). Therefore, any effect of DO level on plant growth should be minimized when comparing the two aeration systems. The pH value with MNB aeration (7.71 ± 0.35) was slightly lower than that with macrobubbles (7.90 ± 0.45) in the second half of the experiment (**Fig. 2c**), but there was no significant difference between the two systems (*P-value = 0.258*).

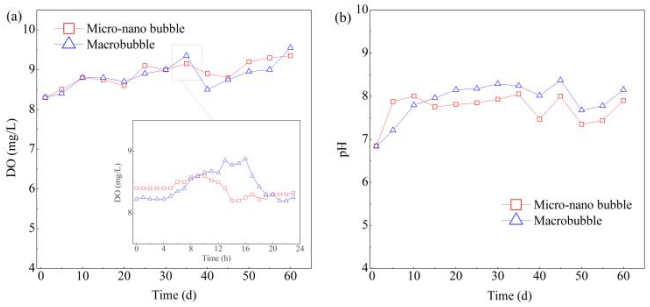

Fig. 2 – (a) The changes of dissolved DO level and (b) pH changes in the nutrient solution during the 60 days of cultivation of EA, EQ and MP (the inset shows the DO level within 24 h).

3.2. Effect of MNB treatment on plant growth

As shown in **Fig. 3**, the growth ratio of fresh weight (i.e., the ratio of the final fresh weight divided by the initial fresh weight), dry weight, the average leaf and root length of EA were all significantly reduced with MNB aeration compared to control plants. The growth of EQ was also inhibited, but there was no significant difference in leaf and root length between the two systems, suggesting that the effect of MNBs on EQ is smaller than that on EA. The third plant species, MP, showed no significant differences between the two conditions. The final fresh weights of EA, EQ and MP in the presence of the same highly concentrated MNBs were 0.75, 0.83 and 1.05 times those in the macrobubble aeration system, which suggests a species-specific effect of MNB aeration.

The chlorophyll content of EA and EQ, especially the former, significantly declined with MNB aeration (**Fig. 4**): the chlorophyll content of EA was almost two-thirds lower after MNB aeration than in plants grown with macrobubble aeration. **Fig. 5** demonstrates the difference in appearance of EA cultured

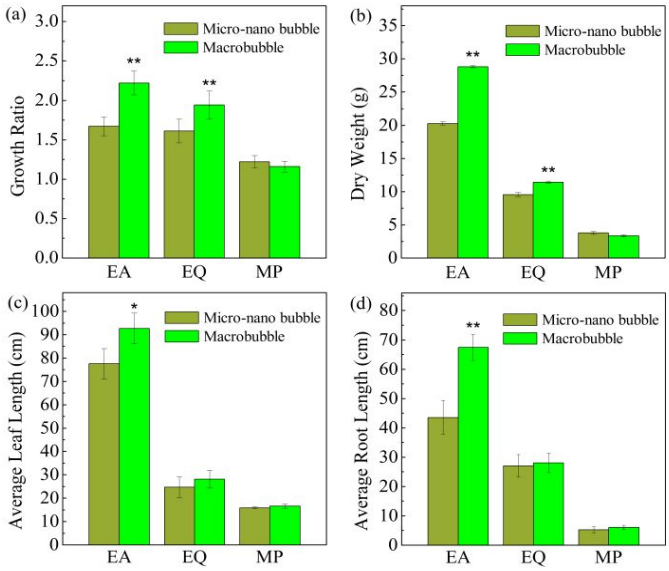

Fig. 3 – Growth ratio of fresh weight (final fresh weight / initial fresh weight), dry weight, average leaf length and average root length of the three plant species over 60 days with aeration by either MNBs or macrobubbles. Vertical bars indicate standard deviation (n = 3).

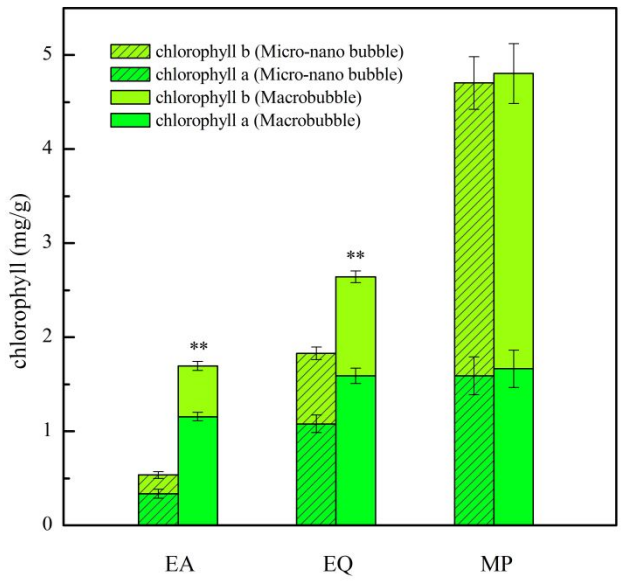

Fig. 4 – The chlorophyll content of EA, EQ and MP plants grown with MNB or macrobubble aeration. Vertical bars indicate standard deviation (n = 3).

with either macrobubble (top left panel) or MNB aeration (top

right). A close-up view of individual leaves is shown in the lower panel. Growth inhibition, yellowing, chlorophyll degradation and in the MNB-aerated examples can clearly be seen in these images.

Fig. 5 - The appearance of EA plants and typical leaves after cultivation with macrobubble (left) or MNB aeration (right).

3.3. Plant senescence analysis

In recent years, a growing body of evidence has shown that ROS can be associated with and regulate plant senescence.[30,31] For example, expression of a plastid-targeted flavodoxin prevents stress-induced ROS formation and delays senescence in aging tobacco leaves,[32] ROS increase during drought-induced leaf senescence in *Arabidopsis*[33] and dark-induced leaf senescence in Pelargonium cuttings[32]. In plants, yellowing and chlorophyll degradation[34], the inhibition of photosynthesis rate, carbon fixation and plant growth[35], and the decline of respiration rate[36] are all symptoms of senescence. In this study, the growth inhibition observed in EA and EQ during MNB aeration was accompanied by several indicators of senescence, such as yellowing and chlorophyll degradation (**Fig. 3**, **Fig. 4, Fig. 5**). In addition, despite the fact that there was no significant difference in the average DO and pH level of the two aeration methods, the DO level in the MNB system was lower than that of the macrobubble system during the daytime (**Fig. 2a**), maybe because the rate of photosynthesis had decreased due to the decline in plant activity. Meanwhile, the DO level at night in the MNB system was significantly higher than that in the macrobubble system (**Fig. 2b**), probably because the respiration rate of plants had also decreased. Meanwhile, the difference of pH value between the two systems was probably because of the decline in carbon fixation. Taken together, our results suggest that exogenous ROS generated by MNBs are responsible for premature senescence of plants EA and EQ, resulting in growth inhibition.

3.4. Oxidative stress and antioxidant capacity in plants

ROS can cause oxidative damage to biomolecules such as proteins, sugars, lipids and nucleic acids,[37] which may induce senescence.[38,39] Ikeura et al. considered ROS generated by MNBs to cause oxidative damage of the root tip during spinach cultivation and thereby to inhibit its growth.[17] Malondialdehyde (MDA) is an indicator of oxidative damage due to lipid peroxidation of polyunsaturated fatty acids by ROS[40] and the degree of oxidative damage can be estimated by quantifying MDA in plant tissues.[41] Plant defenses against the harmful effects of ROS are attributed to the formation of superoxide dismutase (SOD), catalase (CAT), glutathione reductase, vitamin E and vitamin C, which are primary component of plant antioxidant capacity. As a measure of the total ability to combat ROS, the T-AOC values of the three plant species were measured. We also measured SOD activity as a complementary indicator, which represents one of the most important antioxidant defense mechanisms in nearly all living cells exposed to oxygen.

Our results show that there was no significant difference in the MDA content of plants EA and EQ after cultivation in the two aeration systems (**Fig. 6a**). However, the SOD content was significantly lower in MNB-treated plants than that in the macrobubble group (**Fig. 6b**). Meanwhile, the T-AOC content of EA over the cultivation period gradually increased in the macrobubble group from 48.00 U/mL to 64.00 U/mL as shown in **Fig. 7**. Conversely, the T-AOC level of EA decreased from 48.00 U/mL to 39.33 U/mL in the MNB system, which was in line with the decline of SOD content in MNB-treated plants. It means macrophytes use their antioxidant defense mechanisms to prevent oxidative damage by ROS. In addition, the decrease of antioxidant

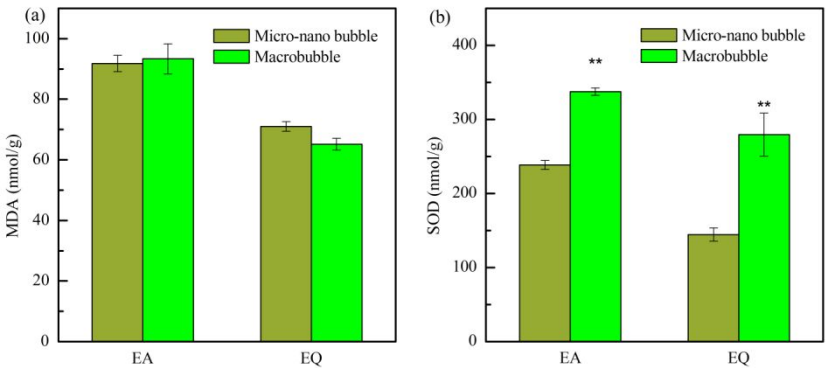

Fig. 6 – The MDA and SOD content of EA and EQ leaves after growth with MNB or macrobubble aeration. Vertical bars indicate standard deviation (n = 3).

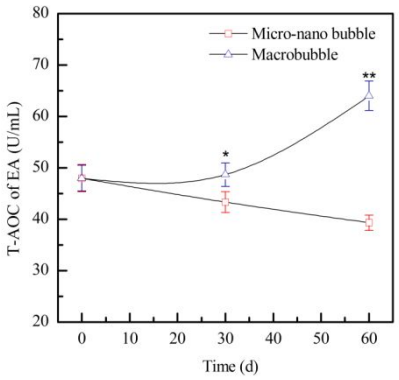

Fig. 7 - The T-AOC of EA during the cultivation period. Vertical bars indicate standard deviation (n = 3).

capacity accords also with the senescence of MNB-aerated plants during cultivation (**Fig. 7**) and the report of Lu and Finkel that lowering antioxidant levels accelerates the onset of senescence.[42]

Intriguingly, when we measured the initial T-AOC content of the three aquatic plant species before the experiment, the T-AOCs of EA and EP were much lower than that of MP, the values being 48.00, 64.67 and 617.33 U/mL, respectively (**Table 1**). The $Ratio_{FFW}$ and other growth indexes (**Table 1**, **Fig. 3**, **Fig. 4**) of each plant were positively correlated with their T-AOC. Thus, we assume that senescence and poor growth were not significant MP because of its high T-AOC that can sequester ROS produced by MNBs. Liu et al. also demonstrated that water containing a high density of NBs had different effects on the germination of spinach seeds and carrot seeds,[15] probably because different plants exhibit tolerances to the same level of ROS due to the differences in the plant T-AOC. In our study, the produced ROS from MNBs might be high enough to include senescence for EA and EQ with low T-AOC. However, on the plant MP, no significant difference in plant growth was observed under the same exposure to the same level of MNBs, because the high content of T-AOC in MP plants sequestered and neutralized the produced ROS from MNBs.

Table 1. Initial fresh weight (IFW), Cultivation condition, Final fresh weight (FFW), Growth ratio = FFW / IFW, $Ratio_{FFW}$ = Final fresh plant weight with MNB aeration/ Final fresh plant weight with macrobubble aeration, and Initial T-AOC of the three plant species.

Plant	IFW (g)	Condition	FFW (g)	Growth ratio	$Ratio_{FFW}$	Initial T-AOC (U/mL)
EA	160.41 ± 4.65	MNB	267.69 ± 10.78	1.67 ± 0.12	0.75 ± 0.06 b	48.00 ± 3.77 b
		Macrobubble	356.43 ± 14.41	2.22 ± 0.15 **		
EQ	62.79 ± 2.91	MNB	101.36 ± 4.55	1.61 ± 0.15	0.83 ± 0.07 b	64.67 ± 6.59 b
		Macrobubble	121.60 ± 5.37	1.94 ± 0.18 **		
MP	26.17 ± 0.95	MNB	32.00 ± 0.95	1.22 ± 0.08	1.05 ± 0.06 a	617.33 ± 30.17 a
		Macrobubble	30.44 ± 0.85	1.16 ± 0.07		

The data are means with standard deviations. One-way analysis of variance was performed with $p < 0.05$ (*) and $p < 0.01$ (**) deemed to be significant. Different letters indicate significant differences according to the Tukey's HSD test ($p < 0.05$).

3.5. Potential mechanisms

ROS are not only a natural byproduct of the normal metabolism of oxygen, but are also important signalling molecules[43,44] regulating growth, development,[45] senescence[30,42] and even programmed cell death.[46,47] It was reported that the generation of low levels of ROS is necessary for the activation of proliferative pathways, supporting plant stem cell renewal and differentiation.[48,49] The growth of lettuce[25,26] and *B. campestris*[28] was also promoted in the presence of low MNBs levels, which means low ROS levels. Under these conditions, the T-AOC of plants is adequate to prevent elevated ROS levels and probably make it act as a positive stimulus to promote plant growth, as illustrated in **Fig. 8.** Meanwhile, when plants are grown under unfavorable conditions such as high-light exposure,[50] drought,[51] chilling,[52] heavy metals,[46] and UV radiation,[53] the high levels of ROS will accumulate inside the plant, which are versatile signalling molecules contributing to stress acclimation.[54] Premature senescence is a protective mechanism employed when plants are stressed and their growth is consequently inhibited.[35,55] It is worth noting that the term "low level" and "high level" are plant type dependent, where the MNB concentration in this experiment was high for EA and EQ but moderate for MP.

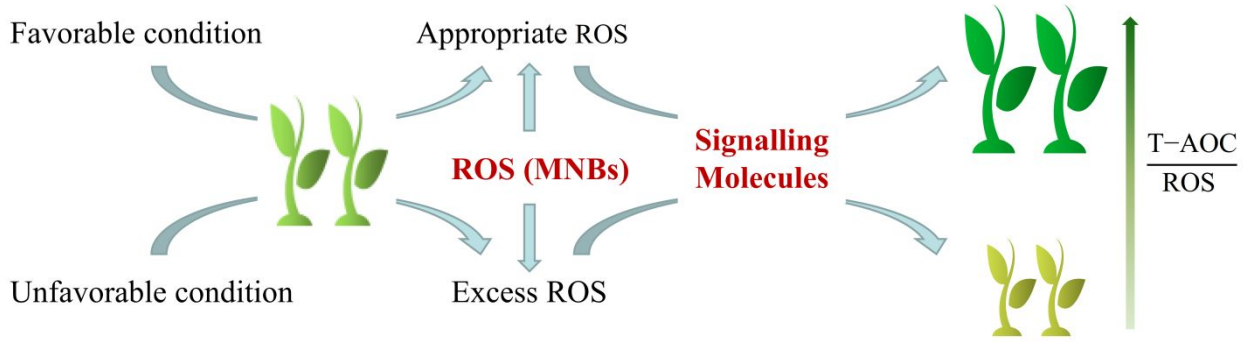

Figure 8 – Potential mechanisms

It was reported that there are 150 genes in *Arabidopsis* whose function is to control endogenous ROS production and scavenging,[45] thereby preventing oxidative damage and allowing ROS to function as signal transduction mediators.[56] Our own results also show that there was no MDA accumulation in MNB-aerated EA and EQ plants (**Fig. 6a**), perhaps due to the effect of SOD and other antioxidants (**Fig. 6b, Fig. 7**), which can protect plants from oxidative damage by ROS, instead allowing ROS to function as signalling molecules. Endogenous ROS are produced in response to environmental conditions, and thus ROS concentration can serve as an indicator of external conditions. The exogenous ROS produced by MNBs probably conveyed incorrect signalling information to plants, "mislead" them into reacting as if this level of endogenous ROS had been produced, and caused a species-specific effect on plant response. For instance, the ROS may promote senescence of macrophytes with low total antioxidant capacity in urban landscape water and thereby affect ecological restoration.

By transforming an external condition into internal biological signals, ROS function as regulatory factors in concert with plant hormones which control almost all aspects of plant growth and development. Several studies have shown that there is an interaction between ROS and the hormone signalling pathways, such as Gibberellins (GAs), ethylene and auxins.[37,57,58] GAs can regulate various developmental processes, including stem elongation, leaf senescence and the transition between vegetative and reproductive growth.[59] Ethylene can accelerate leaf senescence and induce leaf abscission.[60] The high levels of endogenous ROS induced under unfavorable conditions might regulate plant growth in concert with plant hormones, and this probably promotes a shift from the vegetative stage to the reproductive stage, thereby accelerating plant senescence. It is worth noting that senescence does not always play a negative role in plants. The recycling of nutrients to storage organs during the senescence process has a positive effect on production[30], which may be the most optimal response to environmental stress.

In addition, the effects of MNBs on macrophytes were not caused by osmotic stress or nutrients available in water as other papers suggested. (See the discussion in supporting information online)

4. Conclusions

Macrophytes play a vital role in restoring the aquatic ecosystem. This study has demonstrated that MNBs may lead to premature senescence of macrophytes with low total antioxidant capacity, characterised by growth inhibition and a specific senescence phenotype. The exogenous ROS generated by MNBs probably convey false environmental information to macrophytes as signalling molecules and "mislead" the plants into reacting as they would to endogenous ROS. Premature senescence is a protective mechanism employed when plants are stressed and their growth is consequently inhibited. With the extensive application of MNBs in landscape water treatment and ecological restoration, the negative effects on macrophytes should be minimized. Our results indicated that MNBs may negatively affect or damage dense macrophytes and therefore should be applied at controlled doses.

Conflicts of interest

There are no conflicts to declare.

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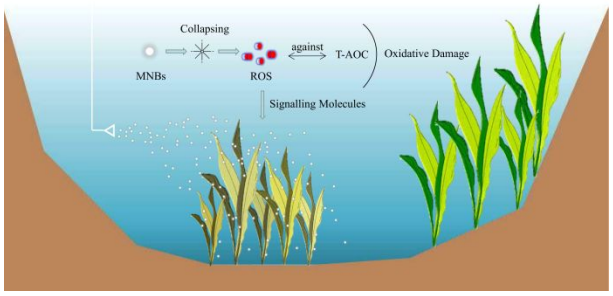
ToC



Reactive oxygen species (ROS) were generated during the collapsing of Micro-nano bubbles (MNBs). Macrophytes cultivated with MNB aeration stimulated antioxidant defenses aimed at preventing oxidative damage by ROS, while allowing exogenous ROS to function as signalling molecules to regulate plant growth, development and senescence. The exogenous ROS produced by MNBs probably conveyed incorrect signalling information to plants, "mislead" them into reacting as if this level of endogenous ROS had been produced, and caused a species-specific effect on plant response. For instance, the ROS may promote senescence of macrophytes with low total antioxidant capacity in urban landscape water and thereby affect ecological restoration.

A table of contents entry:

MNBs may promote senescence of macrophytes with low total antioxidant capacity in urban landscape water and thereby affect ecological restoration.