Supporting Information

For

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

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1. Size distribution of bubbles generated to oxygenate water

We used two complementary techniques to measure the size distribution of bubbles generated by our apparatus in the micro- and nanoscale ranges (see Materials and Methods). After 5 min aeration, the pulse of bubbles was switched off and the MB size distribution was measured over a 60 s interval (at 0s, 5s and 60s; **Fig. S1a**). At each timepoint, the average bubble size decreased from 44.24 μm (CV = 32.44%) to 19.57 μm (CV = 92.64%) to 9.49 μm (CV = 127.52%). After a further 25 min (without bubbling), MBs had disappeared and stable NBs were detected (five replicates) of which 89% were below 200 nm in diameter and 26% below 100 nm (**Fig. S1b**) and the stable MNB concentration was $2.81 \pm 0.45\times10^{7}$ particles/ml. Then the next 30 min cycle of aeration began. Thus, the MBs generated during the pulse of bubbling at the start of the 30 min cycle have apparently mostly collapsed to a relatively stable population of NBs by the end of the cycle.

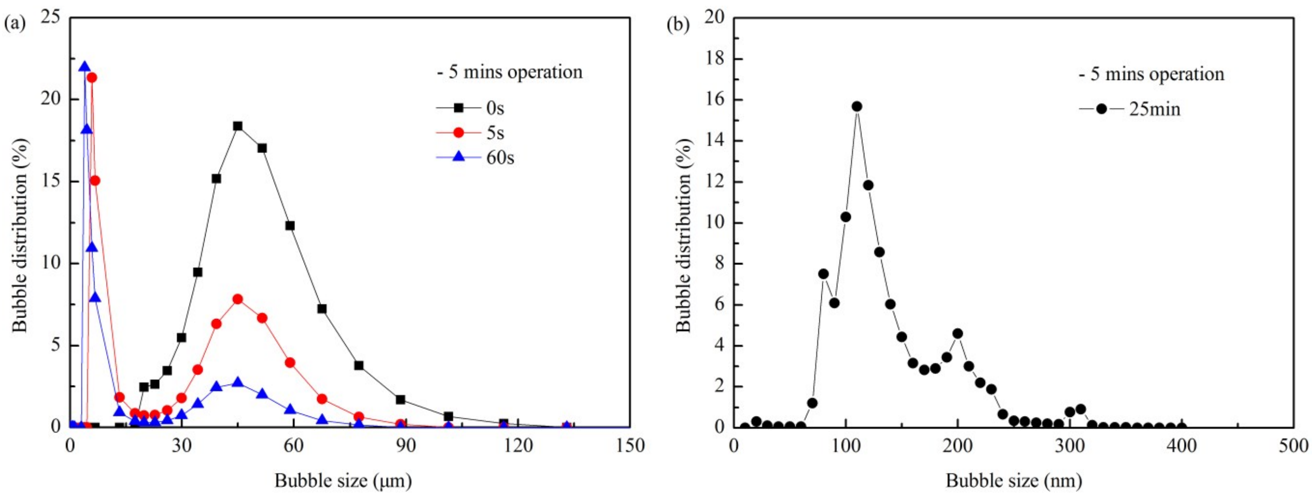

Figure S1. Size distribution of micro- and nanoscale bubbles during a 30 min cycle of aeration.

2. Nitrogen and phosphorus available to plants in water aerated by MNBs and macrobubbles

The concentration of plant-available nitrogen and phosphorus plays an important role in biological processes and plant growth. In our study, the total plant-available nitrogen (NH_4^++NO_3^-) fluctuated and the plant-available phosphorus decreased continuously in both systems during the experimental period, but no significant differences between the two systems were found.

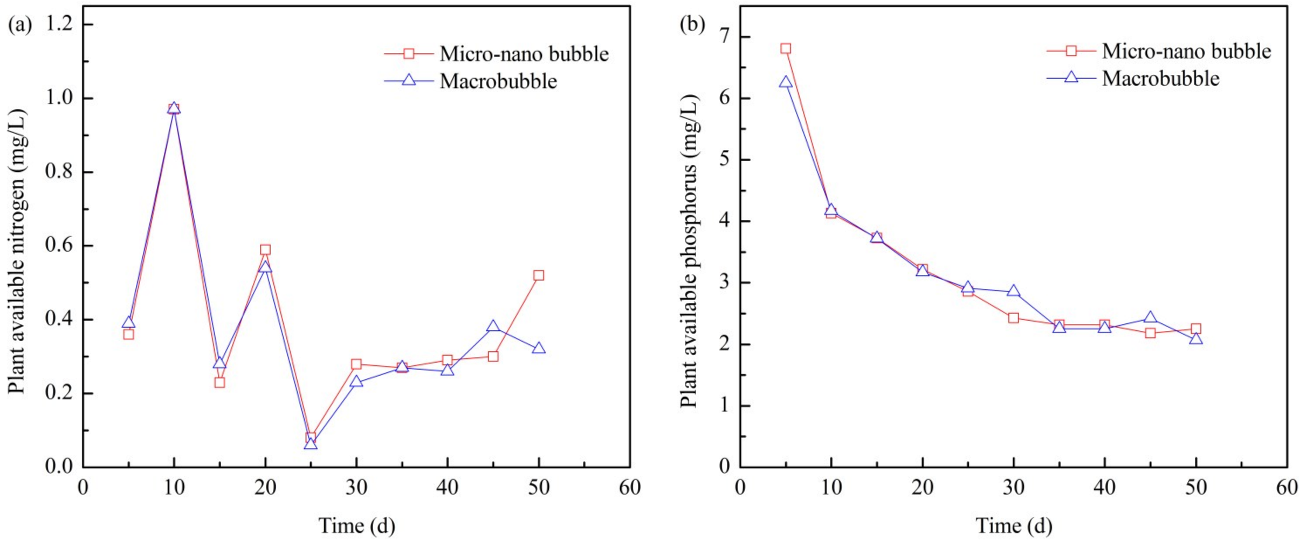

Figure S2. Changes in (a) plant-available nitrogen and (b) plant-available phosphorous in the two aeration systems over the cultivation period.

3. Osmotic pressure and PRO measurement

Due to their long half-life and negative surface charge, MNBs can become attached to plant roots very easily, especially NBs. Park and Kurata suggested that, in this way, MNBs can supply oxygen directly to the roots and also attract positively charged ions, including nutrients dissolved in the growth solution, and are thus beneficial to plants.[1] However, a larger accumulation of positively charged nutrients produced by P-MBs would create a hypertonic nutrient solution around the roots, resulting in reduced osmotic potential and consequent osmotic stress.[2] In this study, the roots of the submerged plants are covered by the soil substitute, while the leaf surfaces are hydrophilic, which prevents MNBs from accumulating on the roots and leaves， thereby eliminate this factor. Nevertheless, if the plants in our experiment experience an increase in external osmotic pressure, they should increase their internal osmotic pressure in response by accumulating compatible solutes such as proline (PRO).[3,4] However, our results show that there was no significant difference in osmotic pressure between the plants in the two aeration systems (**Fig. S3a**). Despite this, the PRO contents of both EA and EQ decreased after cultivation with MB aeration and certainly did not increase (**Fig. S3b**), as suggested by the literature. As PRO is also capable of detoxifying ROS by the formation of long-lived adducts,[5,6] the decline of PRO content (alongside the lower SOD activity) in MNB-aerated plants might be responsible for the reduced T-AOC level in EA over time. We found no evidence that plant growth is promoted by the accumulation of MNBs or inhibited by any associated osmotic stress.

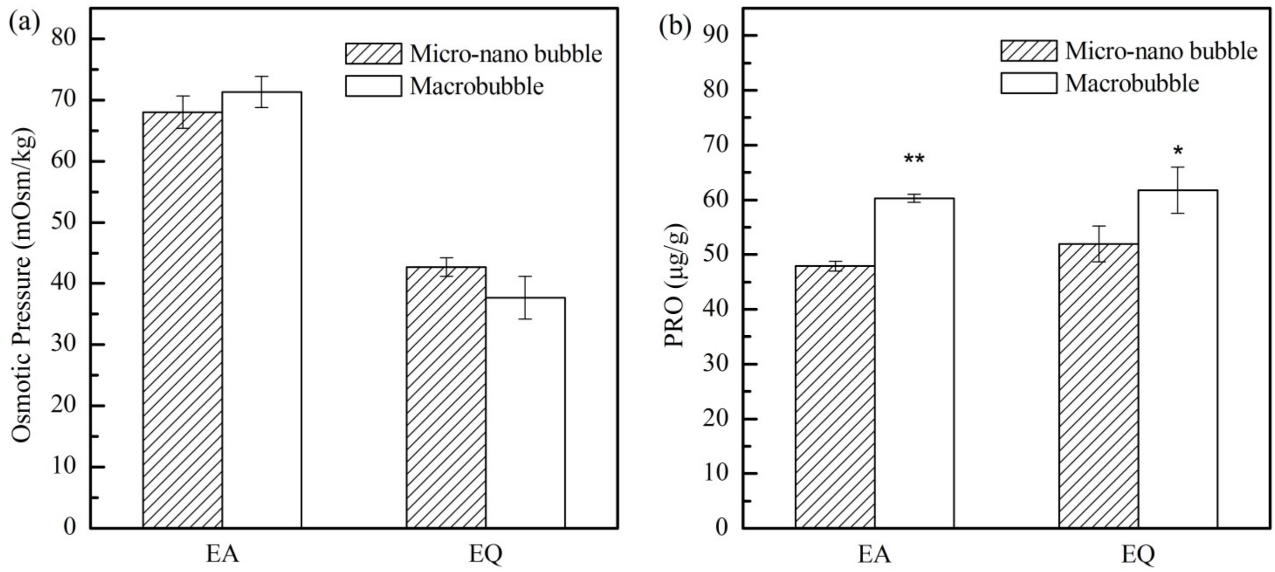

Figure S3 - The relative osmotic potential and PRO content of EA and EQ leaves. Vertical bars indicate standard deviation (n = 3).

4. The appearance of EQ and MP plants

Figure S4. The appearance of EQ plants after cultivation with macrobubble or MNB aeration.

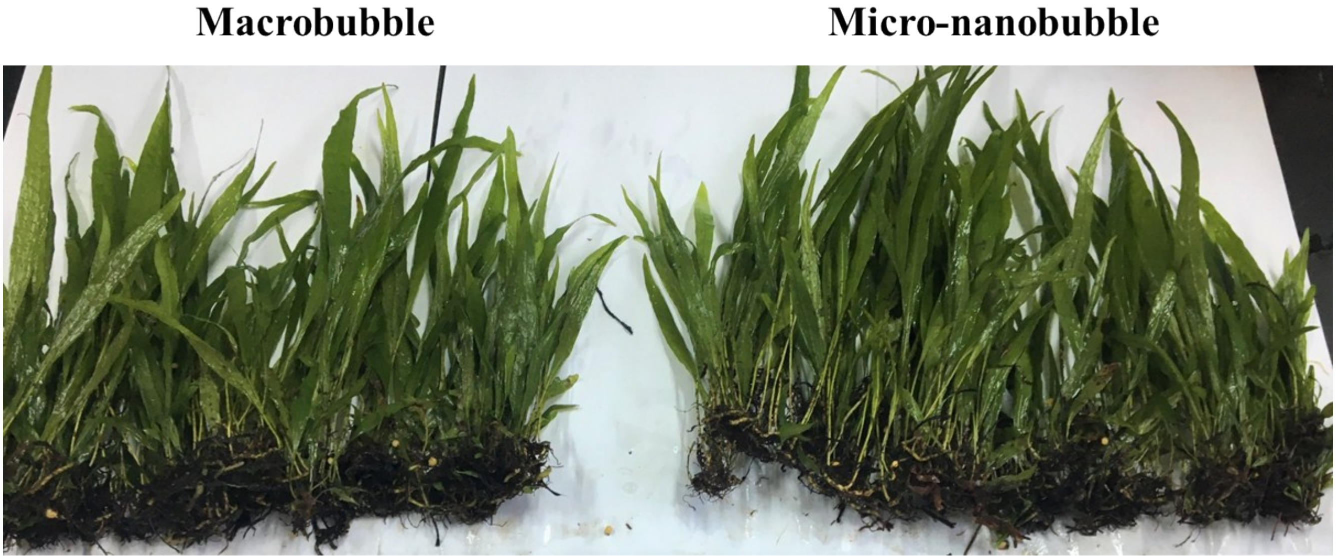

Figure S5. The appearance of MP plants after cultivation with macrobubble or MB aeration.

References

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