Enhancement of Tomato Plant Growth and Productivity in Organic Farming by Agri-Nanotechnology Using Nanobubble Oxygation

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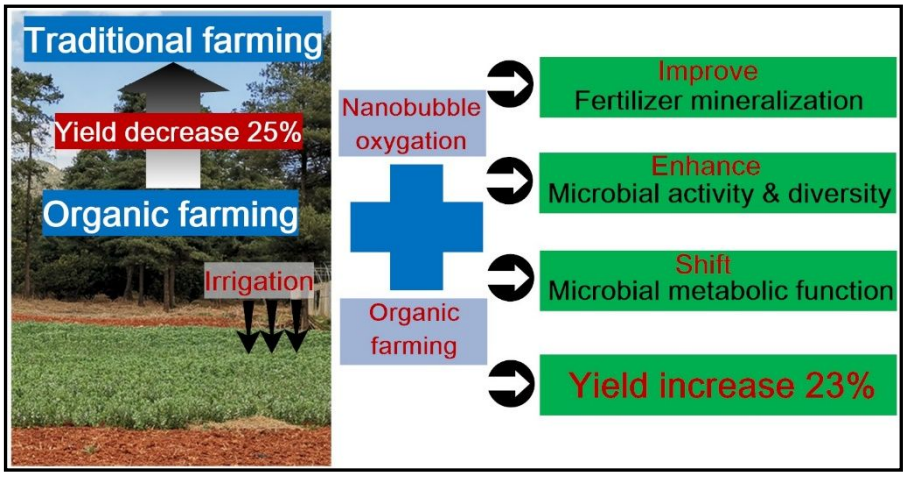
Abstract

Development of technology to improve the mineralization of organic fertilizer and to enhance crop production is essential to achieve the transition from traditional farming to eco-friendly organic farming. Nanobubble oxygation (NB) was employed to compare with traditional pump aerated oxygation (AW) and a control group through both soil incubation and soil column experiments. Plant-available N and P contents in the NB treatment group were higher than that in the AW and control groups. Enzymatic activities including β-1,4-*N*-acetyl-glucosaminidase, phosphatase, α-1,4-glucosidase, β-1,4-xylosidase, peroxidase, and phenol oxidase were significantly higher in both oxygation groups compared with the control. The soil microbial biomass, activity, and diversity were also significantly improved due to the oxygation treatment. Additionally, the microbial metabolic functions were shifted in both oxygation treatments compared with the control group. The final tomato yield increase from the NB treatment group was 23%, and that from the AW treatment 17%, compared with the control.

Keywords: Agricultural sustainability; crop intensification; organic farming; precision farming; oxygen nanobubble

35 **TOC Art**


Traditional farming
Yield decrease 25%
Organic farming
Irrigation
Nanobubble oxygation
Organic farming
Improve
Fertilizer mineralization
Enhance
Microbial activity & diversity
Shift
Microbial metabolic function
Yield increase 23%

1. Introduction

Currently, an estimated 124 million people in 51 countries are facing crises from food insecurity and shortage based on the 2018 report by UN's World Food Programme (WFP). One of the greatest challenges is how to increase 50 percent of food production to ensure that the growing global population - predicted to be around 10 billion by 2050 - has enough food to meet their nutritional needs. Many techniques and approaches have been developed in order to improve crop growth and yield, a simple approach being to increase the application of chemical fertilizer in the traditional farm.[1] However, increased fertilizer usage on farmland can cause groundwater pollution,[2] surface water eutrophication,[3] and nutrient loss[4] though runoff or leaching. To avoid the adverse impact on both environment and ecosystem,[5] it is essential to develop an eco-friendly approach for the enhancement of agricultural crop production.

Organic farming is an ideal environmentally-friendly agricultural system, which relies on organic fertilizers derived from livestock manure, crop residues or human excreta.[6] Organic farming also strives for sustainability by promoting natural pest control and minimising environment pollution from synthetic pesticides and antibiotics.[7] However, in organic farming, the applied nutrients from the organic fertilizer can only be utilized by crops after decomposition and mineralization of organic matter and release of plant-available nutrients, such as nitrogen and phosphorous. It has been reported that only 35%, 39%, and 53% of the plant-available nitrogen can be released from cow, pig and chicken manures on farmland over 6 months, respectively.[8] As a result, crop production in organic farming has been demonstrated to be up to 25% lower than that in conventional agriculture using chemical

58 fertilizer.[9] This slow release of mineral nutrients from organic fertilizer has become the major
59 yield-limiting factor,[10] which indicates that further research could focus on the acceleration of
60 the mineralization of organic fertilizer in organic farming.

61 The mineralization is driven by microbial biodegradation processes, where oxygen is
62 crucial in order to improve the bio-decomposition rate. The soil oxygen content in traditional
63 farmland originates mainly from air diffusion, which is always limited, especially in the deep
64 soil layer. Thus, an appropriate method to deliver sufficient oxygen into the soil is crucial to
65 improve microbial activity. The application of aerated water to the farmland through a drip
66 irrigation system has been used to deliver oxygen to the crop root zone.[11] Previous studies
67 demonstrated that these approaches could not only enhance crop yields, but could also
68 improve the nutrition quality of fruit.[12] To improve the soil oxygenation efficiency, the
69 aeration pump was upgraded from common air pumps, fine bubble diffusers and to venturi
70 injectors.[13] The main aim of the development of this technique was to deliver smaller-sized
71 air bubbles into irrigation water and to improve oxygen dissolution efficiency. Recently,
72 nanobubble technology (NBs; defined as bubbles with diameters less than 1000 nm,[14, 15] has
73 attracted increasing attention due to characteristics of high gas solubility and long lifetime of
74 oxygen in the liquid.[3, 16] The use of a mixture of micro- and nano- bubbles has been used for
75 the oxygation in drip irrigation systems for water saving and for increasing vegetable yields.[17]
76 Air, oxygen and nitrogen saturated nanobubble waters, used for irrigation, have been
77 demonstrated to improve the yield of such plants as lettuce, and seed germination and
78 biomass growth.[18, 19] However, the effect of the nanobubble technology on the mineralization
79 of organic fertilizer still need to be demonstrated.

Previous studies have mainly focused on the effects of oxygation on plant physiology, crop yield, quality, and water use efficiency. Soil oxygation can directly improve the plant root growth and nutrient uptake by providing required oxygen for root respiration and energy generation.[20] However, evaluating the effect of oxygation on soil properties is also important in order to reveal the mechanisms for crop yield enhancement. It has been proven that soil microbial structure, activity and metabolic functions in the soil could be altered, associated with the change of soil oxygen content.[21] Moreover, enzyme activity in soil is important as it directly influences biochemical processing of soil nutrients.[22] Therefore, studying the metabolic functioning of the microbial community, and soil enzyme activity, coupled with the mineralization of organic fertilizer after the oxygation treatment, can help us better understand the mechanisms of altered crop growth.

To evaluate the effect of the proposed nanobubble oxygation method on organic fertilizer mineralization and crop growth, the tomato plant and cow manure compost were selected as the model crop and target organic fertilizer, respectively. Firstly, a soil incubation experiment was conducted to 1) investigate the effect on organic fertilizer mineralization by monitoring the plant available nitrogen (NH_4^+, NO_3^-) and phosphorus (PO_4^{3-}); 2) evaluate the influence on soil enzymes activities related to C-, N-, and P-cycling; 3) detect the response of the metabolic functioning of the soil microbial community. Secondly, a soil column experiment was set up to 4) study the hypothesized positive effect of nanobubble oxygation on tomato growth and yield. From the results, this study aimed to demonstrate a promising agri-nanotechnology, nanobubble oxygation, for the improvement of crop yields in organic farming.

2. Materials and methods

2.1 Aerated water and nanobubble solution preparation

The oxygen nanobubble aerated water was generated by a nanobubble generator (KTM, Nikuni Co., Ltd., Kanagawa, Japan). Briefly, the generator was operated by recirculation of a fixed volume of 20 L deionized water at a flow rate of 1000 L/h. The superficial liquid velocity in the column was 0.035 m/s, and the residence time in the system was approximately 2.1 min. Pure oxygen (>99%) and air (v/v=1:1) were injected into the system under the gas flow of 0.45 L/m. The system was run for 5 min before use, and the dissolved oxygen (DO) of the irrigation water was approximately 15 mg/L measured by a DO meter (HQ40d, HACH, USA). In order to set a comparable irrigation water as the traditional oxygation treatment, pure oxygen and air (v/v=1:1) was used to aerate 20 L deionized water under the gas flow of 0.45 L/m. The aeration was stopped after approximate 5 mins when the DO of aerated water reached 15 mg/L under the directly measurement by a DO meter (HQ40d, HACH, USA). Thus, the gas volume used for nanobubble solution and aerated water solution were both around 2.25 L.

2.2 Soil incubation experiment

A laboratory-scale soil incubation experiment (Fig. 1a) was performed in order to investigate the combined effects of oxygation and organic fertilizer application in the soil. Two treatment groups were designed as 1) cow manure applied soil, irrigated by nanobubble aerated deionized water (NB) and 2) cow manure applied soil, irrigated by traditional aeration deionized water (AW). A control group was set as 3) cow manure applied soil irrigated by original deionized water (Control). Before the incubation experiment, the cow manure compost was mixed and passed through a 2 mm sieve. The sieved cow manure compost was

mixed with 600 g of the topsoil at a rate of 1.5% then placed in 1 L transparent plastic jars. The soil was compacted to give a bulk density of 1.3 g cm^{-3}. The jars were covered with loose lids to allow air circulation but to minimize water evaporation. There were twelve replicates jars in each treatment group and the incubation lasted for 28 days in an illuminated incubator with constantly dark environment at 25 °C. During the incubation period, all soil jars were maintained at 65% field water-holding capacity. The weight loss of each jar was checked every two days, and corresponding irrigation water was added to maintain a constant soil moisture content.

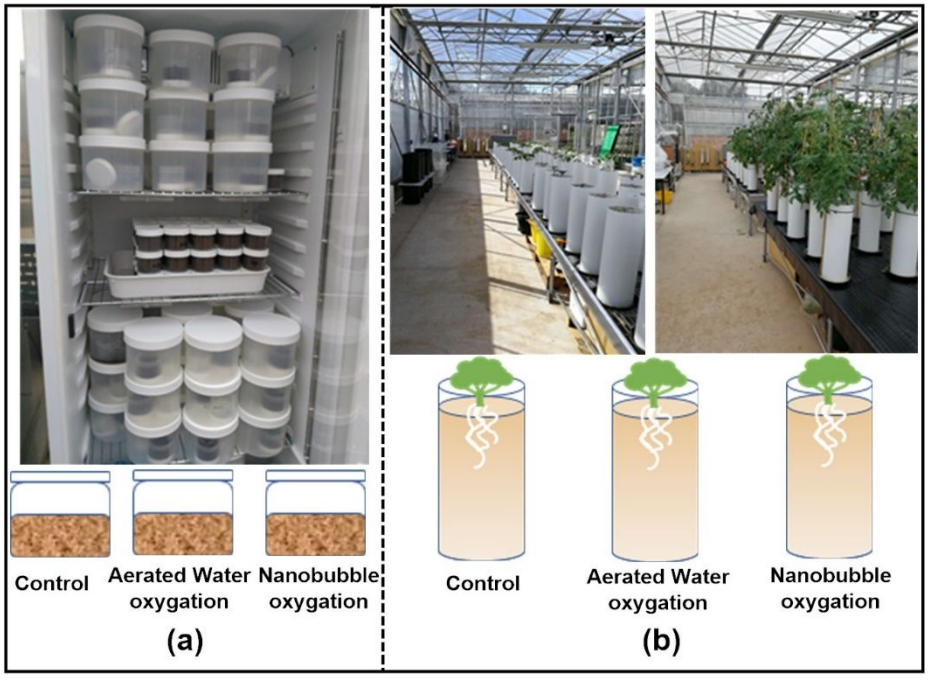


Fig. 1. Schematics and photos of the lab-scale soil incubation (a) and soil column (b) experiments.

2.3 Soil column experiment for tomato growth

To evaluate the effect of combined oxygation and organic fertilizer application on tomato biomass growth and yield, a greenhouse soil column experiment was performed (Fig. 1b). The experiment was conducted from 1st July to 8th September in 2018 in the greenhouse

at Brackenhurst campus, Nottingham Trent University, UK. The three experimental groups were designed as follows: 1) the control group (Control, original deionized water + cow manure compost), 2) the aerated water oxygation treatment group (AW, normal bubble aerated water + cow manure compost), and 3) the nanobubble oxygation treatment group (NB, oxygen nanobubble aerated water + cow manure compost). In each group, 12 replicated, planted, soil columns were prepared. Each soil column was 25 cm high with a diameter of 15 cm. Topsoil (0-20 cm, 29% sand, 42% silt, and 29% clay), collected from Embleys Farm in the UK, was air dried and sieved by 2 mm mesh. Then, 5 kg of topsoil was mixed with 75 g of cow manure compost before filling the columns. The same size of tomato seedlings at the 3 to 4 leaf stage were then transplanted into each pot. The plants were watered every day during the experiment to maintain 65 % field water-holding capacity.

2.4 Sampling and analysis

2.4.1 Nanobubble analysis

The sizes (<1000 nm) and distributions of nanoscale bubbles in the traditional aerated and nanobubble aerated deionized waters were determined by nanoparticle tracking analysis by ZetaView PMX 120 (Particle Metrix, Meerbusch, Germany) and its corresponding software ZetaView 8.04.02. The samples were collected after 5 mins of preparation and the analyses carried out at room temperature. Each sample was analysed with a flow cell sensitivity of 70% across two cycles of 11 positions/cycle.

2.4.2 Sampling strategies

For the soil cultivation experiments, soil samples were collected at day 4, 12, 17 and 28

158 during the experiment. The soil in three replicated jars from each treatment group were
159 collected after homogenization by mixing with a glass rod. After sifting the soil samples
160 through a 2-mm sieve, the soil was air-dried prior to the determination of plant-available
161 nutrients, N and P. At day 28, each soil sample was divided to three parts for nutrient analysis
162 (part I) and the determination of dissolved organic carbon (DOC) and microbial biomass
163 carbon (part II). The remainder of the soil samples (part III) were used to analyse the soil
164 enzyme activity and microbial community metabolic functions. For the soil column experiment,
165 the diameter of stem, and the height of tomato plants was recorded at 15, 30, 45 days. Tomato
166 fruit from each treatment was harvested and weighed at day 70.

167 2.4.3 Soil chemical properties

168 The plant-available nitrogen (NH_4^+-N, and NO_3^--N) in soil samples was extracted by 2 M
169 KCl solution according to the method described by Tu *et al.,* (2006).[23] Plant-available
170 phosphorus (PO_4^{3-}-P) was extracted with 0.5 M $NaHCO_3$ following a previously-reported
171 method.[24] The concentrations of NH_4^+-N, NO_3^--N, and PO^{3-}-P in the extracts were determined
172 by analysis on an AQ400 nutrients auto-analyzer (Seal Analytical, Southampton, UK). The
173 chloroform fumigation-extraction method was used to determine the microbial biomass
174 carbon (MBC). The dissolved organic carbon (DOC) content of the extract was measured by a
175 Shimadzu TOC-V Total Organic Carbon Analyser (Shimadzu Corp., Kyoto, Japan).

176 2.4.4 Soil enzyme activities

177 In the soil cultivation experiment, the hydrolytic enzymes, peroxidase, phenol oxidase,
178 α-1,4-glucosidase, and β-1,4-xylosidase were selected as indicators for C acquisition.[25] The

terminal reaction in chitin degradation can be catalyzed by β-1,4-N-acetyl-glucosaminidase, thus it was evaluated as one of the N-targeting hydrolytic enzymes.[26] Phosphatase is the enzyme responsible for releasing labile inorganic P for microbes and plants.[27] The activities of all extracellular enzymes, except for phenol oxidase and peroxidase, were measured by using the MUB-linked model substrate method described by Zhao et al., (2016).[28] The phenol oxidase and peroxidase activities were measured spectrophotometrically by using L-3,4-dihydroxy-phenylalanine as the substrate in a clear 96-well microplate.

2.4.5 Microbial metabolic functions

In the soil cultivation experiment, community-level physiological profiling (CLPP) of the soil samples were assessed by using Biolog EcoPlate™ (Biolog Inc., California, USA).[29] A 1000-fold serial dilution of the rhizosphere soil suspension was made and 150 µl aliquots were added to each well in the microplates. Soil particles were not removed, nor allowed to settle, during any step in the extraction or inoculation. The plates were then packed into polyethylene bags to reduce evaporation and were incubated in the dark at 25 °C. Absorbance at 590 nm was measured on an automated microplate reader (Tecan Group Ltd. Austria) after 24, 48, 72, 96, 120, 144 and 168 of incubation hours. Each well absorbance value was corrected by subtraction of the optical density of a control well. The CLPP data was analysed, based on the previous studies,[30, 31] to calculate the average well colour development (AWCD) and Shannon diversity indexes.

2.5 Statistical analyses

The data were assessed with one-way ANOVA. Duncan's multiple-range test was applied

200 when one-way ANOVA revealed significant differences ($p<0.05$). All data were tested for a
201 normal distribution and variance homogeneity using Levene's test. The statistical analyses
202 were performed with SPSS ver. 13.0 statistical software (SPSS, Chicago, IL, USA). In addition, a
203 Principal Components Analysis (PCA) was performed on correlation matrix of CLPP results
204 using Origin Pro 2016 software (OriginLab Corp., Massachusetts, USA). For PCA analysis, data
205 were standardized by autoscaling method prior to analysis to ensure that each variable had
206 the same influence in the analysis.

207 ## 3. Results

208 ### 3.1 Nanobubbles distribution in oxygenated waters

209 The aerated waters prepared for irrigation were analysed in the nanoparticle-tracking
210 analysis instrument to detect the size and distribution of the nanobubbles (Fig. 2). The
211 concentration of nanobubbles (<1000 nm) was 4.1×10^7 particles/mL in the aerated irrigation
212 water prepared by the traditional pump (Fig. 2a), however, a one-magnitude higher
213 nanobubble concentration (7.5×10^8 particles/mL) was observed in the nanobubble-aerated
214 water after 5 mins operation (Fig. 2b), with 87% below 200 nm in diameter. It should be noted
215 that the deionized water before any aeration treatment contained undetectable
216 concentrations of nanobubble ($<10^4$ particles/mL; data is not shown).

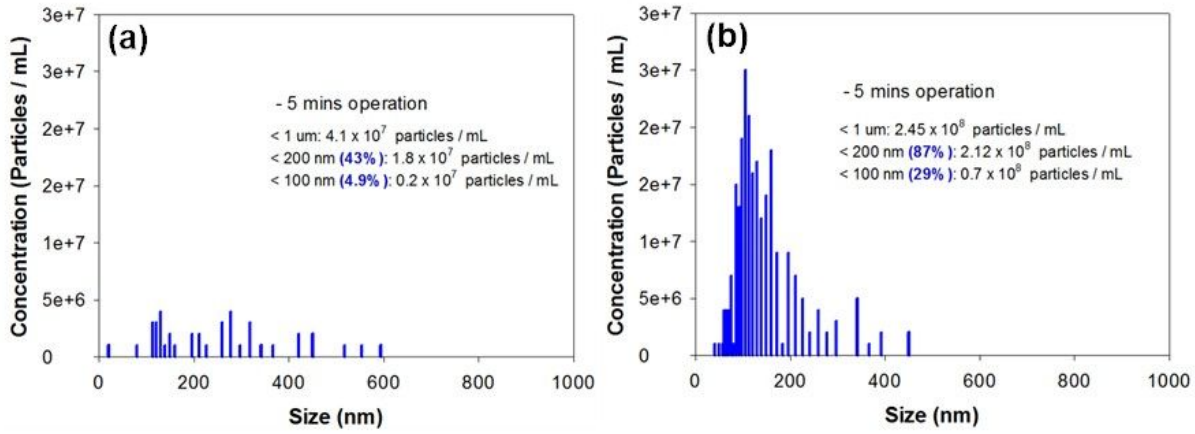


Fig. 2. Nano-scale bubbles size and distribution in traditional aerated (a) and nanobubble aerated (b) solutions, measured by NTA.

3.2 Plant-available nutrients and organics

The total plant-available N (NH_4^+ + NO_3^-) content generally increased from around 38 mg/kg to 50, 56, and 66 mg/kg at day 28, in the control, AW treatment and NB treatment groups, respectively (Fig. 3a). Compared with the control group, enhancements of 12% and 32% total available N were observed in the AW and NB treatment groups, respectively. It can be noted that the concentrations of NH_4^+ in all three groups remained at a similar level (around 11 mg/kg) throughout the experiment. The significantly higher NO_3^- content in the soil is the main contribution for the enhancement in total N content. A similar tendency in plant-available P in the soil was detected in all groups throughout the experiment, where the soil from NB treatment group contained significantly higher P concentrations (5.9 mg/kg), followed by AW treatment (4.9 mg/kg) and control groups (4.4 mg/kg) (Fig. 3b). No significant differences in the concentrations of dissolved organic carbon (DOC, Fig. 3c; range from 67.0-79.5 g/kg) were found in the three groups at the end (day 28) of the incubation. The amount of microbial biomass carbon (MBC) was significantly affected by oxygation treatment (Fig. 3d).

In the AW and NB treatment groups, MBC concentrations were significantly increased by 17% and 26%, respectively, compared to the control treatment.

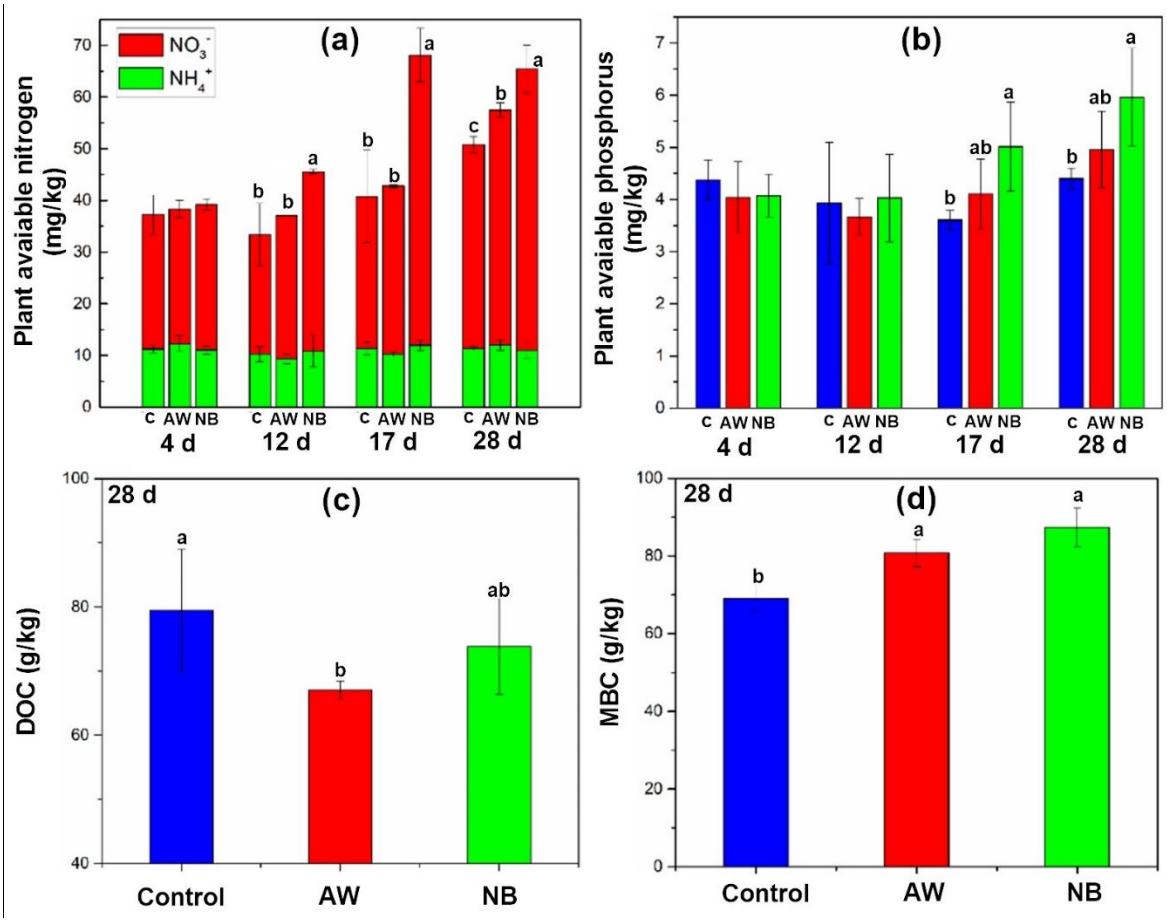


Fig.3. Effect of oxygation on plant-available nutrients, i.e. (a) nitrogen and (b) phosphorus, (c) dissolved organic carbon (DOC), and (d) microbial biomass carbon (MBC) in soil incubation experiments. Control: irrigation with original water, AW: irrigation with traditional pump-aerated water, NB: irrigation with nanobubble-aerated water. Different letters above the bars in each figure indicate significant difference ($P<0.05$) between three groups in the same sampling day.

3.3 Soil enzyme activities

After the soil incubation experiment, soil enzyme activities were analysed to understand the mechanisms of nutrient mineralization. All six enzymes exhibited higher activities in the

soil samples from the oxygation (AW or NB) treatment groups (Fig. 4). The activities of N-mineralization related enzyme, β-1,4-N-acetyl-glucosaminidase (Fig. 4a), and P-mineralization related enzyme, Phosphatase (Fig. 4b), were significantly higher in the oxygenated groups than the control. There was no significant difference between the NB and AW irrigation groups in the enzyme activity, though for both enzyme activity was higher than for the control group. For the C-cycling related enzymes, the oxygation treatments slightly improved the activities of α-1,4-glucosidase (Fig. 4c), β-1,4-xylosidase (Fig. 4d), and phenol oxidase (Fig. 4f) compared with the control groups. Both AW and NB treatment significantly improved the peroxidase activity compared with the control samples, however, there was no significant difference between them (Fig. 4e).

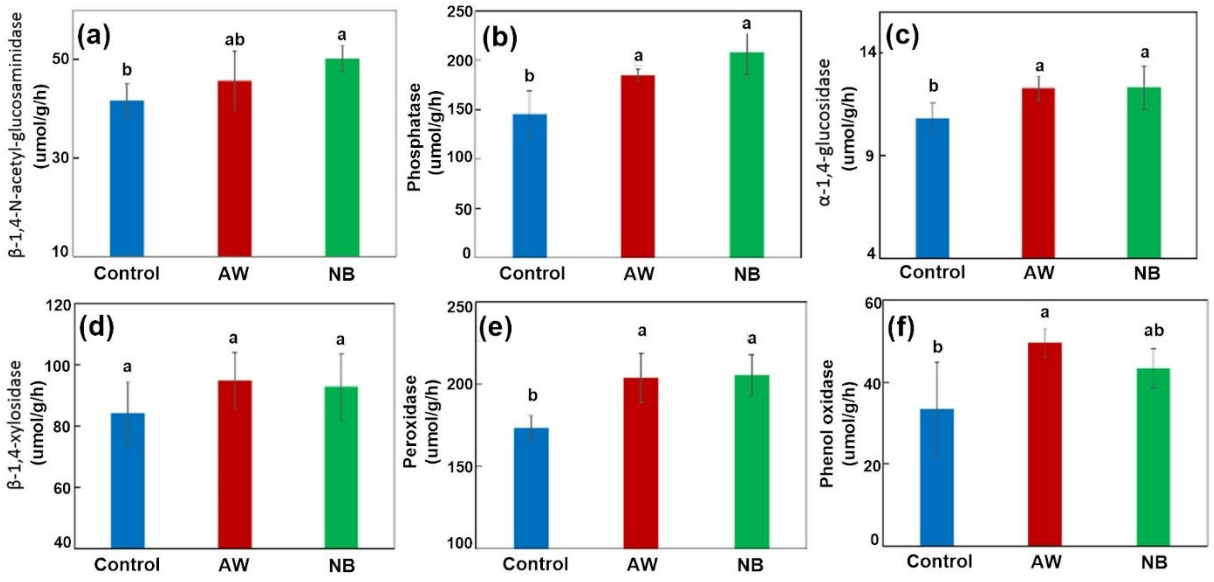


Fig. 4. Effect of oxygation on soil enzyme activities: (a) β-1,4-N-acetyl-glucosaminidase, (b) Phosphatase, (c) α-1,4-glucosidase, (d) β-1,4-xylosidase, (e) Peroxidase, and (f) Phenol oxidase. Control: irrigation with original water, AW: irrigation with pump aerated water, NB: irrigation with nanobubble aerated water. Different letters above the bars in each figure indicate significant difference between the values ($P<0.05$).

3.4 Response of microbial metabolic functions

The community-level physiological profiling was assessed to evaluate the response of the microbial metabolic functions to the NB irrigation. The soil microbial diversity and activity were reflected in the Shannon diversity index and the AWCD values, respectively. Both of these were significantly higher in the oxygation treatment groups compared to the control group (Table 1). The levels of microbial diversity and activity were similar between NB and AW treatment groups.

Table 1

The microbial metabolic functional diversity and average well colour development (AWCD) level from the Biolog EcoPlate™ analysis in control, pump-aerated water (AW) and nanobubble-aerated water (NB) irrigation groups.

Group	Shannon diversity (H')	AWCD (590 nm)
Control	3.344 ± 0.003^{b}	2.0 ± 0.1^{b}
AW	3.387 ± 0.001^{a}	2.4 ± 0.1^{a}
NB	3.388 ± 0.005^{a}	2.5 ± 0.1^{a}

Different superscript letters beside the number indicate a significant difference at $P < 0.05$.

The biochemical properties of the 31 carbon sources in the microplates were organized into six groups (guilds), miscellaneous, carbohydrates, polymers, carboxylic acids, amino acids and amines/amides, in order to reduce the complexity of the data obtained. Overall, the capabilities of the soil microbial communities for carbon utilization were strengthened in the oxygation treatment groups (Fig. 5a). However, only amino acids showed significantly higher

utilization in AW and NB treatment groups than in the control group. Further evaluation was used to transform the multivariate vectors into two uncorrelated principal component vectors (Fig. 5b). The two-dimensional PCA of the community-level of physiological profiles explained 68.6% of the total variance, with the first principle component having a greater power of separation (42.7%). The data from the oxygation treatment groups located in the upper right section of the figure, were significantly different from the control group, shown on the left of the plot. The analysis of the loading of carbon sources on PC1 showed that AW and NB treatments were indeed factors that influenced the catabolic diversity of microbial communities. The data between NB and AW groups are generally overlain (Fig. 5b).

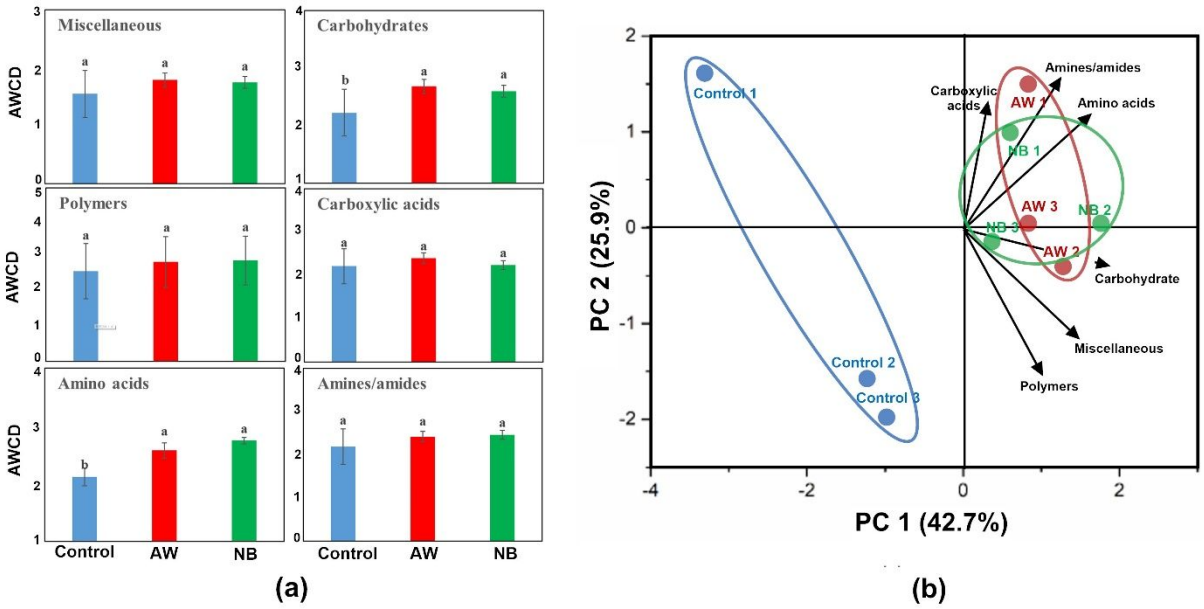


Fig. 5. Microbial community carbon source utilization levels (a) and the principal component analysis (PCA) ordination of the carbon source utilization patterns (b) from Biolog Ecoplates incubated for 168 h. Control: irrigation with original water, AW: irrigation with pump aerated water, NB: irrigation with nanobubble aerated water. Different letters above the bars in each figure indicate significant difference between the values ($P<0.05$).

3.5 Tomato growth and yield

The soil oxygation treatments from both AW and NB significantly improved the tomato growth as measured by improved stem diameter (Fig. 6a) and plant height (Fig. 6b) at the early stage of plant growth on day 15 and 30. However, by day 45, these differences were not significant in both AW and NB treatment groups compared with the control group (Fig. 6a and b). The tomato biomass from the NB oxygation group yielded a significantly higher value (around 547 g/plant) than that (around 447 g/plant) from the control group, an enhancement of some 22% (Fig. 6 c and d). The tomato yield from AW oxygenation treatment group was around 523 g/plant, which was 17% higher than the control group.

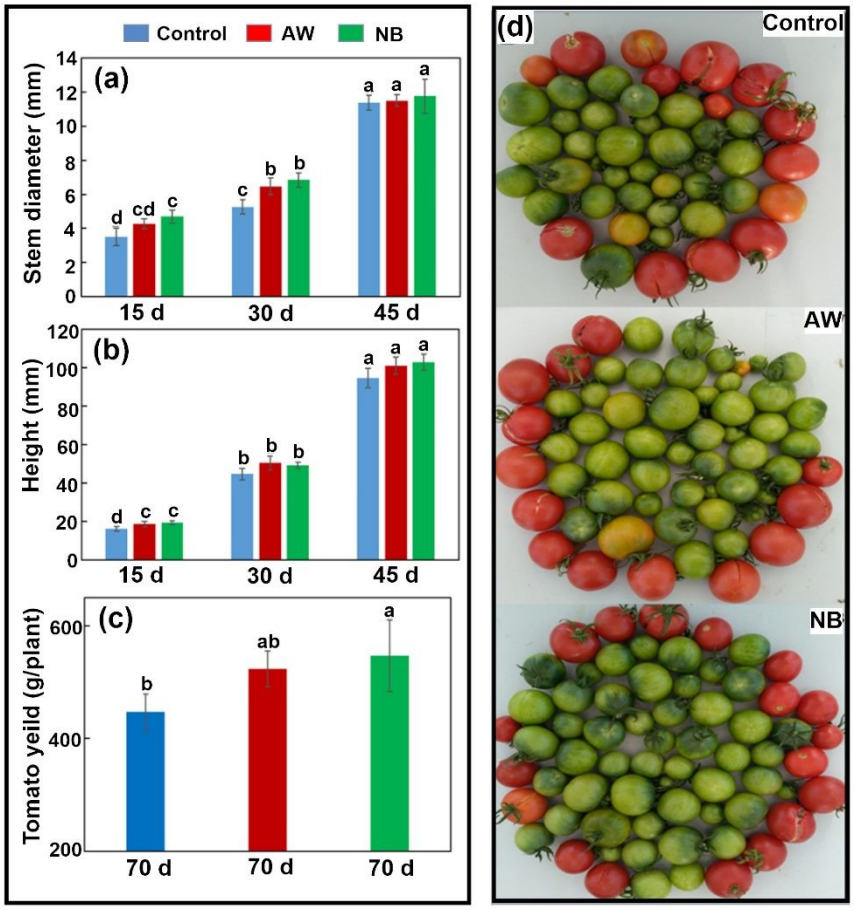


Fig. 6. Tomato plant growth, i.e. (a) stem dimension and (b) plant height, and (c, d) tomato biomass yield at the end of the soil column experiment. Control: irrigation with original water, AW: irrigation

305 with pump aerated water, NB: irrigation with nanobubble aerated water. Different letters above the
306 bars in each figure indicate significant difference between the values ($P<0.05$).

307 4. Discussion

308 In the process of agricultural production, fertilization and irrigation are key practices for
309 improving the yield of crops.[32] To reduce environmental issues caused by overuse of chemical
310 fertilizers without influencing crop yield, organic fertilizer has been recommended as a partial,
311 or even complete, substitute.[33] Oxygation (aerated irrigation) is an irrigation technology which
312 is well recognized to enhance crop yield by improving the aerobic environment of the root
313 zone and to increase root uptake of water and nutrients.[34] However, the study focused on the
314 effect of both soil oxygation and organic fertilizer application on crop growth is still limited. In
315 the present study, two oxygation methods, irrigation by traditional pump-aerated water and
316 nanobubble-aerated water, were applied in growth of tomatoes with organic fertilizer.

317 The majority of the nutrients, stored in organic form in organic fertilizers, cannot be
318 directly utilized by the crops. The release of plant-available nutrients, such as N and P, from
319 organic matter involves biological decomposition processes, which are highly dependent on
320 the oxygen content and moisture level in the soil. Oxygation offers soil sufficient water and
321 oxygen at the same time, thus the plant-available N and P content can be increased, such as
322 occurs under ventilation treatment.[35] It supports the present finding that the irrigation of both
323 normal pump-aerated and nanobubble-aerated waters significantly increased the release of
324 plant-available N and P from organic fertilizer (Fig. 3). Specifically, nitrogen content organic
325 fertilizer can release NH_4^+ through the biodegradation process under the aerobic condition. If

326 the oxygation approach substantially supply the oxygen, NH_4^+ can be transformed to plant
327 available NO_3^- through nitrification process.[36] Thus, the substantially higher content of NO_3^-
328 compared with NH_4^+ (Fig. 3a), may be due to the dominant nitrification process under such an
329 aerobic environment. Moreover, the significantly higher nutrients under NB oxygation may be
330 attributable to the large amounts of nanoscale bubbles in NB-aerated irrigation water (Fig. 2).
331 The NB has a low buoyancy and long lifetime, where the filled air or oxygen can be slowly
332 dissolved into the soil interstitial water and sustainably supply the oxygen[37] required for the
333 mineralization of organic fertilizer. The effective oxygen supply by NBs may also result the high
334 speed of the organic fertilizer mineralization and plant-available N in the soil achieved the
335 highest value in day 17 (Fig. 3a). Similar level was shown in day 28 may cause by the thoroughly
336 plant-available N release under the oxygation treatment. It is differentiated from the normal
337 pump-aerated water, where the oversaturated oxygen can escape from the irrigation water
338 quickly to the atmosphere resulting in a comparatively reduced oxygenation effect and speed
339 on the rhizosphere environment.

340 Agriculture practices, such as irrigation, can influence soil microenvironment and result
341 in the shift of soil microorganisms.[38] Higher soil aeration was reported to stimulate microbial
342 biomass and change community composition in paddy fields,[39] findings which support the
343 determination of improved microbial biomass, activity and diversity in the aerated irrigation
344 groups in this study (Fig. 3d and Table 1). The differences of microbial metabolic functions in
345 the soil samples were indicated by the utilization of 31 kinds of carbon sources during the
346 Biolog microplate analysis.[40, 41] The clearly differentiated metabolic function groups between
347 the aerated irrigation group and the control (Fig. 5), further demonstrated that the

348 oxygenation treatments not only boosted microbial activity, but also played a constructive
349 role in increasing functional diversity of soil microbial communities.[42] Even though the
350 microbial metabolic functions (Fig. 5b) were undifferentiated between the normal pump-
351 aerated and nanobubble-aerated irrigation treatments, gene level differences in the soil
352 microbial communities may be significant, which need to be further studied.

353 Soil extracellular enzymes are mainly synthesized and secreted by soil microorganisms.[43]
354 Changes in metabolic function and diversity of soil microbial community might cause the
355 fluctuation of soil enzyme activities. Previous studies found some soil enzyme activities were
356 greater in soils treated by aeration than in those without.[35, 44] In this study, we found soil
357 enzyme activities were increased by oxygation treatment. The mechanism may be due to the
358 stimulation of microbial growth and the increase in the activity of the extracellular enzyme-
359 organo complex.[45] Among the 6 enzymes we measured (Fig. 4), the activities of C-cycling
360 enzymes (α-1,4-glucosidase, β-1,4-xylosidase, phenol oxidase and peroxidase), a N-cycling
361 enzyme (β-1,4-N-acetyl-glucosaminidase) and a P-cycling enzyme (Phosphatase) suggest a
362 shift toward increased C acquisition as N and P becomes readily available for plant growth.
363 Increases in enzyme activities may reflect and stimulate soil microbial activity, thereby
364 increasing the quantities of nutrients available to plants.[46] However, the similar enzymes
365 activities were observed in the two oxygation treatments, which may due to the relative short
366 soil incubation time before the sampling.

367 Plant height and stem diameter were significantly increased in the early stage of tomato
368 plant growth (15, 30 days) in both oxygation treatments (Fig. 6). The result is consistent with

previous studies showing that oxygation treatment can improve the rate of organic fertilizer mineralization and result in a fast crop growth.[47] However, the plant growth (stem diameter and height) achieved the same level at the final stage after the fruit had ripened (Fig. 6). Similar results were also found for the tomato cultivation under aerated irrigation.[12] which may due to the same amount of fertilizer application in all groups. It has been reported that the tomato yield with oxygation treatment was around 19% higher when compared to non-oxygation treatment.[48] In the present study, the AW treatment with traditional pump-aerated irrigation reached a similar increase (17%) of tomato production, while the nanobubble-aerated irrigation achieved around a 23% improvement in yield (Fig. 6 c), which is comparable to the losses (up to 25%) generally attributed to the transition from traditional farming using chemical fertilizer to organic farming using organic fertilizer.[9] Therefore, the present study provides a promising eco-friendly agri-nanotechnology, with which to increase crop production in organic farming. Nevertheless, further study should be conducted to evaluate the effect of NB oxygation on organic fertiliser mineralization and crop growth directly in the soil column experiment before the application. Notably, in the present study, the plant-available nutrients and microbial communities from the nanobubble irrigation treatment are only slightly different to those obtained by conventional pump-aerated irrigation group. The relatively larger tomato yield may also be due to the synergistic functions of improvement in organic fertilizer mineralization and plant physiology modification by the nanobubbles.[18, 19] Thus, the plant gene alteration and fruit nutrition changes will need to be further studied.

In conclusion, the nanobubble oxygation treatment for organic farming was evaluated for the improvement of organic fertilizer mineralization and tomato production, compared

with the traditional pump-aerated oxygation technique and with un-oxygenated control groups. Levels of plant-available N and P were substantially improved, associated with the stimulation of soil enzymatic activity due to the oxygation treatment. Moreover, this treatment, in an organic farming context, significantly enhanced the soil microbial biomass, activity, diversity, and metabolic functionality. Even through the differences between the nanobubble oxygation and tradition pump-aerated oxygation treatments were not always significant, final tomato yields improved by approximately 23%, while the pump-aerated oxygenation treatment gave an improvement in crop yield of 17%, when compared to the control group. The results indicated that the proposed agri-nanotechnology, nanobubble oxygation, is a potentially promising approach to stimulate mineralization of organic fertilizer and thus improve crop growth, during a transition from using chemical fertilizer to organic fertilizer for organic farming.

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