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

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Wu Y., Lyu T., Yue B., Tonoli E., Verderio E., Ma Y., et al. (2019). Enhancement of Tomato Plant Growth and Productivity in Organic Farming by Agri-Nanotechnology Using Nanobubble Oxygenation. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, 67(39), 10823-10831 [10.1021/acs.jafc.9b04117].

Availability:

This version is available at: <https://hdl.handle.net/11585/741043> since: 2020-02-28

Published:

DOI: <http://doi.org/10.1021/acs.jafc.9b04117>

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Wu Y.; Lyu T.; Yue B.; Tonoli E.; Verderio E.; Ma Y.; Pan G.: Enhancement of Tomato Plant Growth and Productivity in Organic Farming by Agri-Nanotechnology Using Nanobubble Oxygenation. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 67. 0021-8561

DOI: 10.1021/acs.jafc.9b04117

The final published version is available online at: <http://dx.doi.org/10.1021/acs.jafc.9b04117>

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Yuncheng Wu, Tao Lyu, Bin Yue, Elisa Tonoli, Elisabetta A.M. Verderio, Yan Ma, and Gang Pan

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1 **Enhancement of tomato plant growth and productivity in**
2 **organic farming by agri-nanotechnology using nanobubble**
3 **oxygation**

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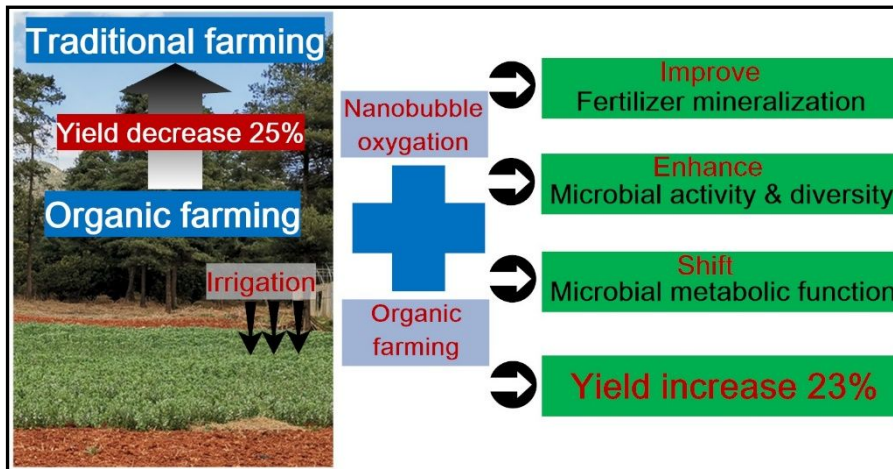
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20 **Abstract**

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

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



36 **1. Introduction**

37 Currently, an estimated 124 million people in 51 countries are facing crises from food
38 insecurity and shortage based on the 2018 report by UN's World Food Programme (WFP). One
39 of the greatest challenges is how to increase 50 percent of food production to ensure that the
40 growing global population - predicted to be around 10 billion by 2050 - has enough food to
41 meet their nutritional needs. Many techniques and approaches have been developed in order
42 to improve crop growth and yield, a simple approach being to increase the application of
43 chemical fertilizer in the traditional farm.¹ However, increased fertilizer usage on farmland
44 can cause groundwater pollution,² surface water eutrophication,³ and nutrient loss⁴ through
45 runoff or leaching. To avoid the adverse impact on both environment and ecosystem,⁵ it is
46 essential to develop an eco-friendly approach for the enhancement of agricultural crop
47 production.

48 Organic farming is an ideal environmentally-friendly agricultural system, which relies on
49 organic fertilizers derived from livestock manure, crop residues or human excreta.⁶ Organic
50 farming also strives for sustainability by promoting natural pest control and minimising
51 environment pollution from synthetic pesticides and antibiotics.⁷ However, in organic farming,
52 the applied nutrients from the organic fertilizer can only be utilized by crops after
53 decomposition and mineralization of organic matter and release of plant-available nutrients,
54 such as nitrogen and phosphorous. It has been reported that only 35%, 39%, and 53% of the
55 plant-available nitrogen can be released from cow, pig and chicken manures on farmland over
56 6 months, respectively.⁸ As a result, crop production in organic farming has been
57 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 oxygenation 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.

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

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

101 **2. Materials and methods**

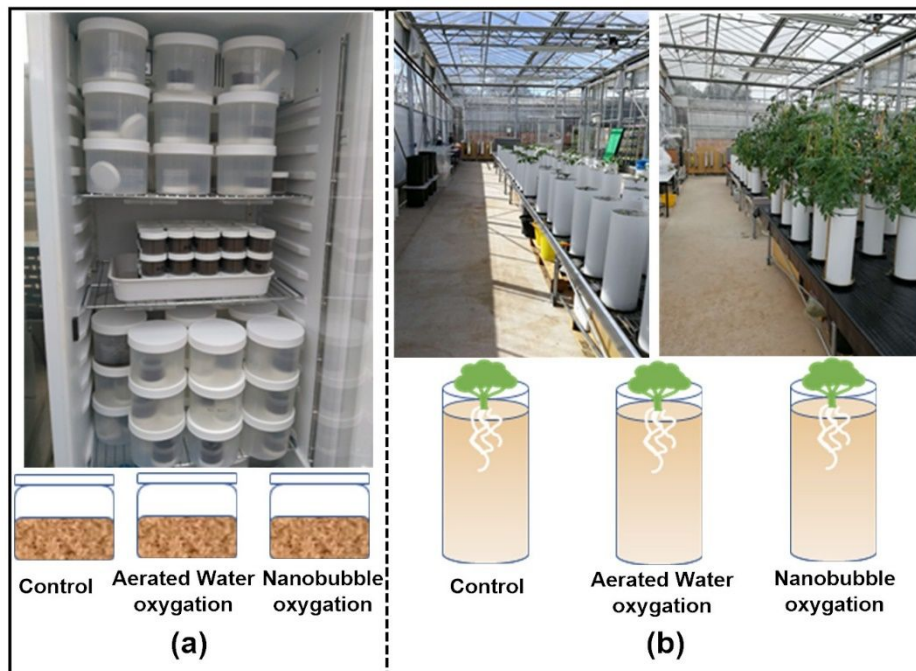
102 **2.1 Aerated water and nanobubble solution preparation**

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

115 **2.2 Soil incubation experiment**

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

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



131
132 **Fig. 1.** Schematics and photos of the lab-scale soil incubation (a) and soil column (b) experiments.

133 2.3 Soil column experiment for tomato growth

134 To evaluate the effect of combined oxygation and organic fertilizer application on
135 tomato biomass growth and yield, a greenhouse soil column experiment was performed (Fig.
136 1b). The experiment was conducted from 1st July to 8th September in 2018 in the greenhouse

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

148 **2.4 Sampling and analysis**

149 2.4.1 Nanobubble analysis

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

156 2.4.2 Sampling strategies

157 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 ($\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$) 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 ($\text{PO}_4^{3-}\text{-P}$) was extracted with 0.5 M NaHCO_3 following a previously-reported
171 method.²⁴ The concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and $\text{PO}_4^{3-}\text{-P}$ 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

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

186 2.4.5 Microbial metabolic functions

187 In the soil cultivation experiment, community-level physiological profiling (CLPP) of the
188 soil samples were assessed by using Biolog EcoPlate™ (Biolog Inc., California, USA).²⁹ A 1000-
189 fold serial dilution of the rhizosphere soil suspension was made and 150 μ l aliquots were
190 added to each well in the microplates. Soil particles were not removed, nor allowed to settle,
191 during any step in the extraction or inoculation. The plates were then packed into
192 polyethylene bags to reduce evaporation and were incubated in the dark at 25 °C. Absorbance
193 at 590 nm was measured on an automated microplate reader (Tecan Group Ltd. Austria) after
194 24, 48, 72, 96, 120, 144 and 168 of incubation hours. Each well absorbance value was
195 corrected by subtraction of the optical density of a control well. The CLPP data was analysed,
196 based on the previous studies,^{30, 31} to calculate the average well colour development (AWCD)
197 and Shannon diversity indexes.

198 2.5 Statistical analyses

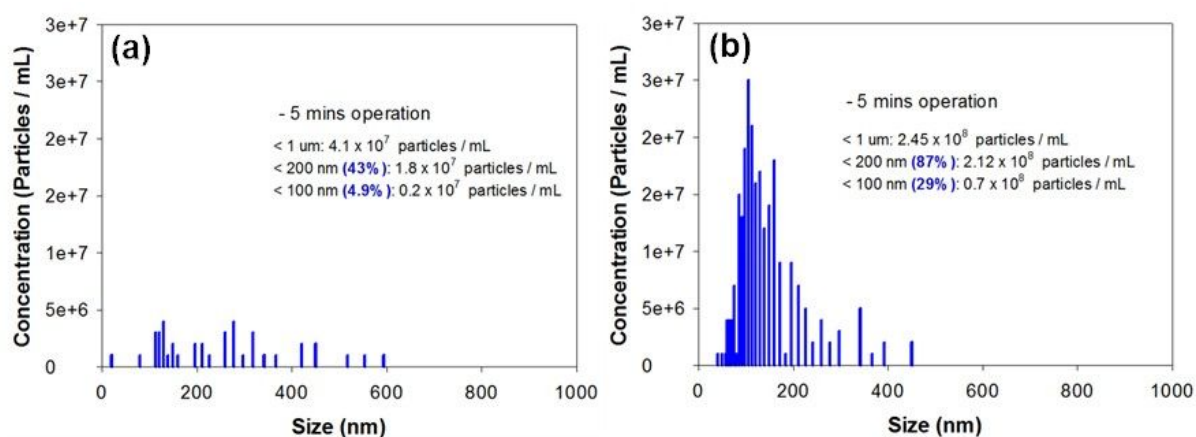
199 The data were assessed with one-way ANOVA. Duncan's multiple-range test was applied

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).

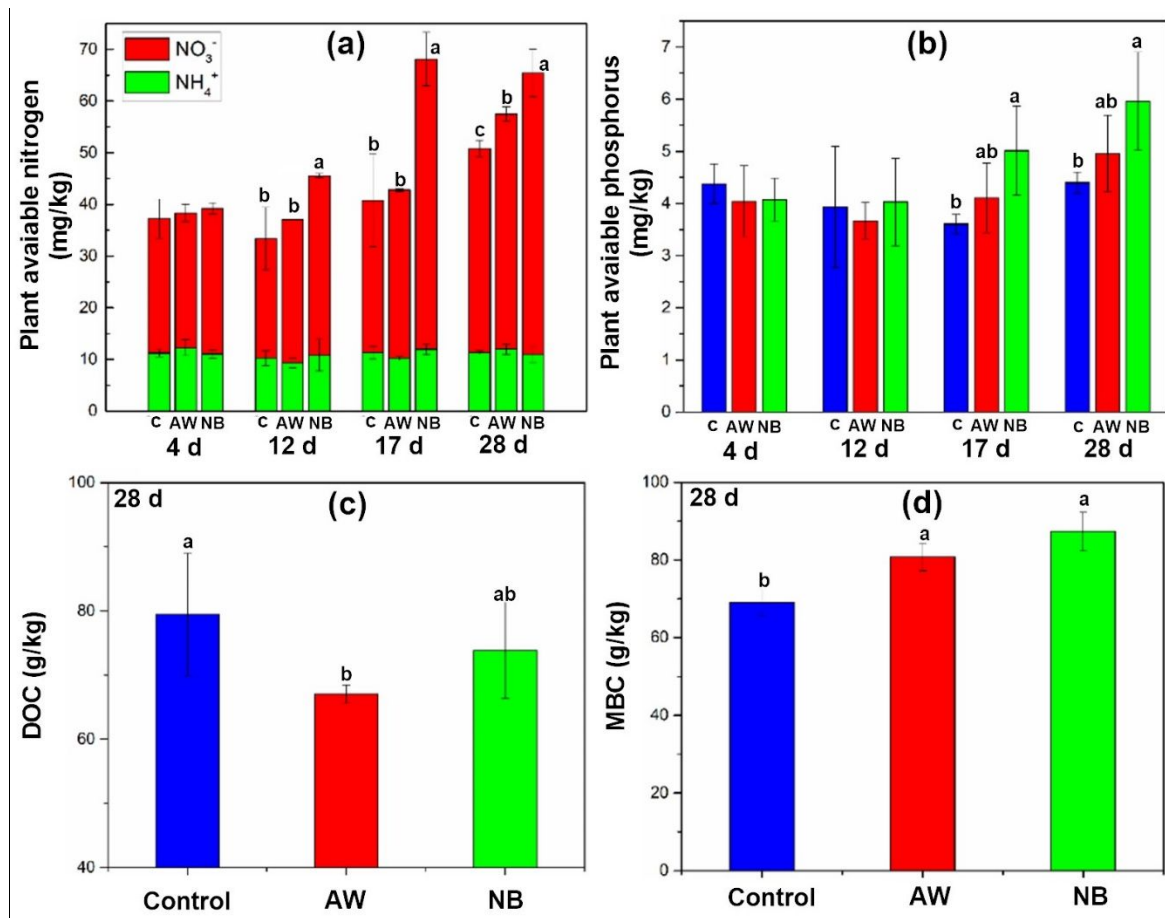


217
 218 **Fig. 2.** Nano-scale bubbles size and distribution in traditional aerated (a) and nanobubble aerated (b)
 219 solutions, measured by NTA.

220 3.2 Plant-available nutrients and organics

221 The total plant-available N ($\text{NH}_4^+ + \text{NO}_3^-$) content generally increased from around 38
 222 mg/kg to 50, 56, and 66 mg/kg at day 28, in the control, AW treatment and NB treatment
 223 groups, respectively (Fig. 3a). Compared with the control group, enhancements of 12% and 32%
 224 total available N were observed in the AW and NB treatment groups, respectively. It can be
 225 noted that the concentrations of NH_4^+ in all three groups remained at a similar level (around
 226 11 mg/kg) throughout the experiment. The significantly higher NO_3^- content in the soil is the
 227 main contribution for the enhancement in total N content. A similar tendency in plant-
 228 available P in the soil was detected in all groups throughout the experiment, where the soil
 229 from NB treatment group contained significantly higher P concentrations (5.9 mg/kg),
 230 followed by AW treatment (4.9 mg/kg) and control groups (4.4 mg/kg) (Fig. 3b). No significant
 231 differences in the concentrations of dissolved organic carbon (DOC, Fig. 3c; range from 67.0-
 232 79.5 g/kg) were found in the three groups at the end (day 28) of the incubation. The amount
 233 of microbial biomass carbon (MBC) was significantly affected by oxygation treatment (Fig. 3d).

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

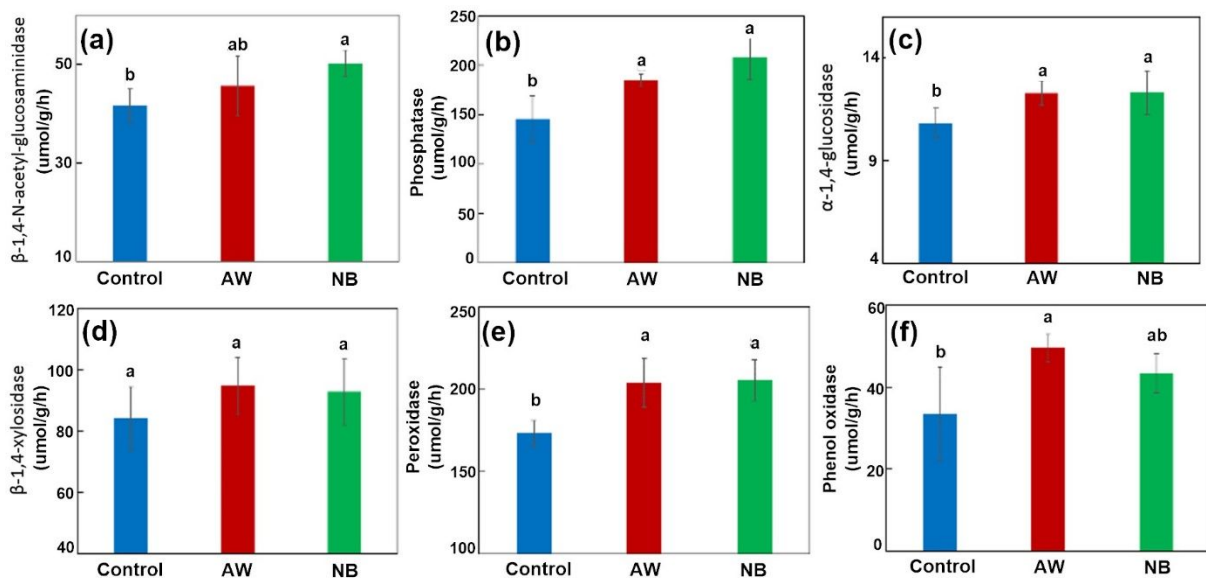


236
 237 **Fig.3.** Effect of oxygenation on plant-available nutrients, i.e. (a) nitrogen and (b) phosphorus, (c) dissolved
 238 organic carbon (DOC), and (d) microbial biomass carbon (MBC) in soil incubation experiments. Control:
 239 irrigation with original water, AW: irrigation with traditional pump-aerated water, NB: irrigation with
 240 nanobubble-aerated water. Different letters above the bars in each figure indicate significant
 241 difference (P<0.05) between three groups in the same sampling day.

242 3.3 Soil enzyme activities

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

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



255 **Fig. 4.** Effect of oxygation on soil enzyme activities: (a) β -1,4-N-acetyl-glucosaminidase, (b)
 256 Phosphatase, (c) α -1,4-glucosidase, (d) β -1,4-xylosidase, (e) Peroxidase, and (f) Phenol oxidase. Control:
 257 irrigation with original water, AW: irrigation with pump aerated water, NB: irrigation with nanobubble
 258 aerated water. Different letters above the bars in each figure indicate significant difference between
 259 the values ($P < 0.05$).
 260

261 3.4 Response of microbial metabolic functions

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

268 **Table 1**

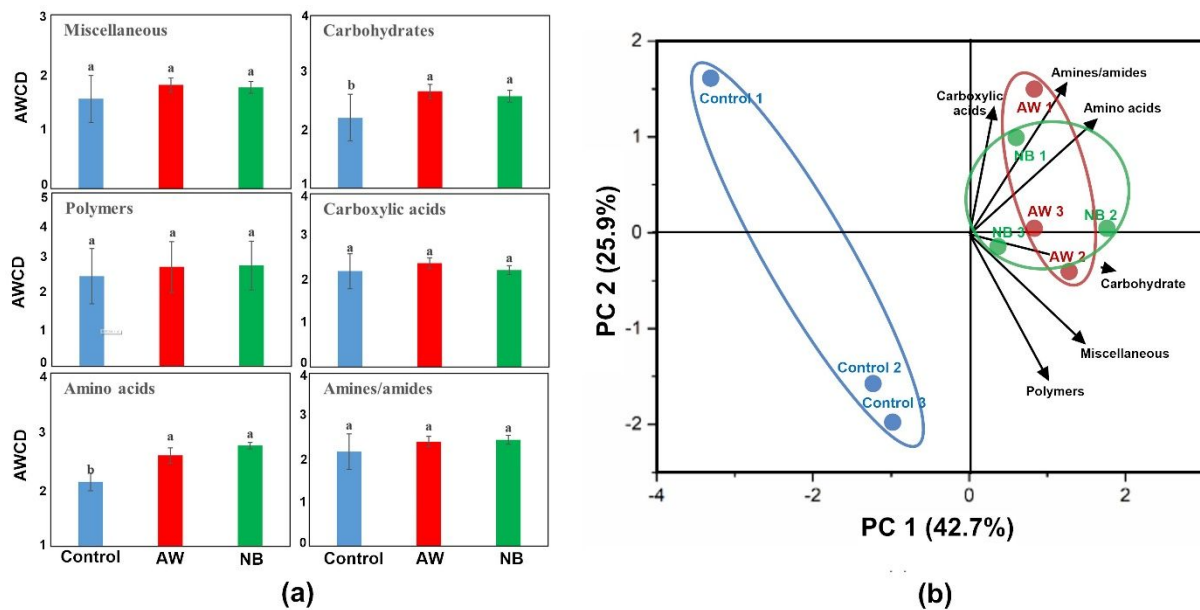
269 The microbial metabolic functional diversity and average well colour development (AWCD) level from
270 the Biolog EcoPlate™ analysis in control, pump-aerated water (AW) and nanobubble-aerated water
271 (NB) irrigation groups.

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

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

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

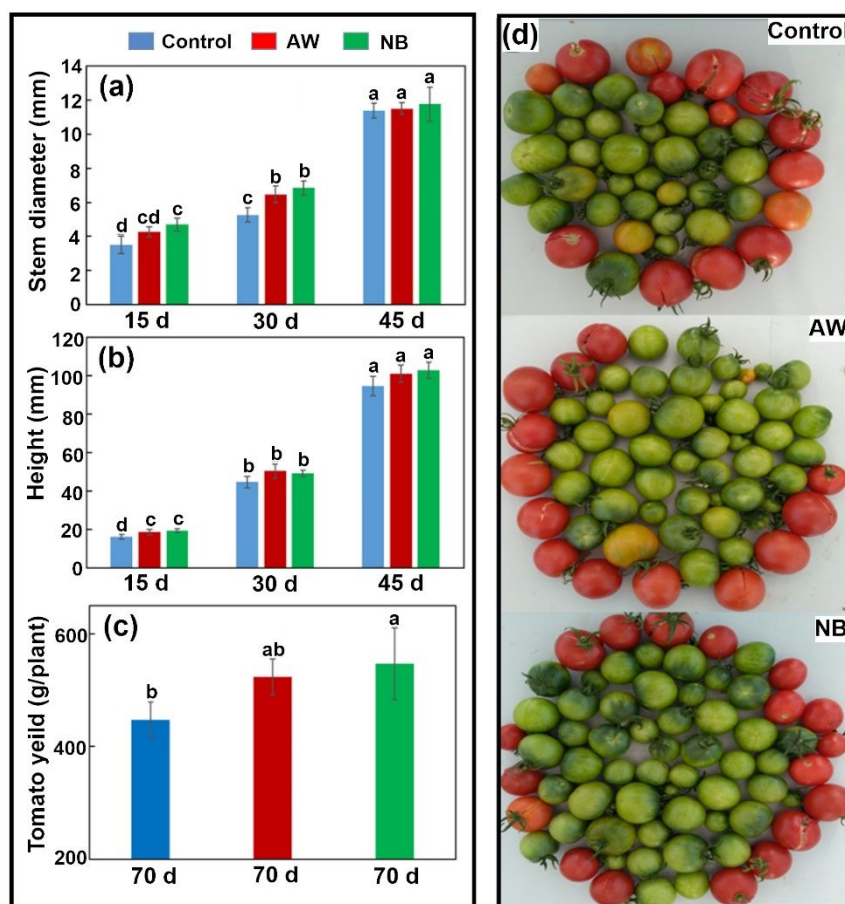
278 utilization in AW and NB treatment groups than in the control group. Further evaluation was
 279 used to transform the multivariate vectors into two uncorrelated principal component vectors
 280 (Fig. 5b). The two-dimensional PCA of the community-level of physiological profiles explained
 281 68.6% of the total variance, with the first principle component having a greater power of
 282 separation (42.7%). The data from the oxygation treatment groups located in the upper right
 283 section of the figure, were significantly different from the control group, shown on the left of
 284 the plot. The analysis of the loading of carbon sources on PC1 showed that AW and NB
 285 treatments were indeed factors that influenced the catabolic diversity of microbial
 286 communities. The data between NB and AW groups are generally overlain (Fig. 5b).



287 **Fig. 5.** Microbial community carbon source utilization levels (a) and the principal component analysis
 288 (PCA) ordination of the carbon source utilization patterns (b) from Biolog Ecoplates incubated for 168
 289 h. Control: irrigation with original water, AW: irrigation with pump aerated water, NB: irrigation with
 290 nanobubble aerated water. Different letters above the bars in each figure indicate significant
 291 difference between the values ($P < 0.05$).
 292

293 **3.5 Tomato growth and yield**

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



302
 303 **Fig. 6.** Tomato plant growth, i.e. (a) stem dimension and (b) plant height, and (c, d) tomato biomass
 304 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 oxygen³⁷ 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 oxygenation 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 oxygenation 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 oxygenation treatments (Fig. 6). The result is consistent with

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

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

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

403 **Acknowledgments**

404 This work was funded by the Ministry of Science and Technology 973 project (No. 2015
405 CB150500), China Postdoctoral Science Foundation (2017M621672), National Natural Science
406 Foundation of China (41701339) and Medical Technologies and Advanced Materials Strategic
407 Theme at Nottingham Trent University. We thank Mick Cooper for proof reading.

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