Title: Fluctuations in the abundance of ammonia-oxidizers and the contents of inorganic nitrogen in solarized soil

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25

Abstract

Solarization makes a great impact on the abundance of ammonia-oxidizers and nitrifying activity in soil. To elucidate fluctuations in the abundance of ammonia-oxidizers and nitrification in solarized soil, copy numbers of *amoA* gene of ammonia-oxidizing bacteria and archaea, viable number of ammonia-oxidizers and inorganic nitrogen contents were investigated in greenhouse experiments. The copy number of *amoA* gene and the viable number of ammonia-oxidizers were determined by the quantitative polymerase chain reaction and most probable number methods, respectively. Abundance of ammonia-oxidizing bacteria based on the estimation of *amoA* gene copy numbers and viable counts of ammonia-oxidizers was decreased by the solarization treatment and increased during the tomato (*Solanum lycopersicum* L.) cultivation period following the solarization. Effect of solarization on the copy number of *amoA* gene of ammonia-oxidizing archaea was less evident than that on ammonia-oxidizing bacteria. The proportion of nitrate in inorganic nitrogen contents was declined by the solarization and increased during the tomato cultivation period following the solariztion. Positive correlations were found between the proportion of nitrate in inorganic nitrogen content and the copy number of bacterial or archaeal *amoA* gene or the viable number of ammonia-oxidizers; the copy number of bacterial *amoA* gene showed a strong correlation with the viable number of ammonia-oxidizers. The present study revealed influences of solarization on the fluctuation in the abundance of

ammonia-oxidizers and dynamics of inorganic nitrogen contents in soil and the results indicate that the determination of *amoA* gene of ammonia-oxidizing bacteria is possibly a quick and useful diagnostic technique for evaluating suppression and restoration of nitrification following solarization.

Key words: Ammonia-oxidizing bacteria, ammonia-oxidizing archaea, nitrification, quantitative PCR, solarization

1. Introduction

Soil disinfection techniques consist of chemical methods using agrochemicals and physical methods mainly by heating. The physical methods contain steam sterilization, hot water sterilization and solarization (Kita 2006). Solarization is an environment-friendly and cost-effective soil disinfection technique, which is an alternative to soil fumigation with methyl bromide.

High soil temperature achieved by the solarization for the disinfestation of plant pathogens and pests (Katan 1981; Stapleton 2000) makes a great impact on soil microorganisms and their functions besides pathogenic microorganisms. It is well known that soil disinfection treatments inhibit the nitrification process, which supplies nitrate to plants. Rapid recovery of nitrification following soil disinfection is important to prevent growth retardation of crops by ammonium accumulation in the disinfested

66 soil (Morikuni *et al.* 1999; Nishihara 2008). As for the solarization, inhibition and
67 subsequent recovery of nitrificationin soil have been already reported (Nishihara 2008;
68 Wada *et al.* 2008; Ihara *et al.* 2014). Many studies reported a profound inhibition of
69 nitrification concomitant with great decrease in the viable counts of ammonia-oxidizers
70 estimated by the most probable number (MPN) method by soil disinfection. Subsequent
71 restoration of the nitrification was associated with recovery of the viable counts of
72 ammonia-oxidizers (Suzuki and Watanabe 1966; Suzuki *et al.* 1968; Tagawa and
73 Tomaru 1969, Takei *et al*. 1974; Kato *et al*. 1984; Morikuni *et al.* 1999). Close
74 relationships between the viable number of ammonia-oxidizers estimated by the MPN
75 method and nitrifying activity of soil after soil sterilization have been well documented
76 (Watanabe 1974). The inhibition and recovery of nitrifiers were evaluated based on the
77 viable counts by the MPN method (Nishihara, 2008) and the nitrifying activity (Wada *et*
78 *al.* 2008; Ihara *et al.* 2014), respectively.

79 Ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea
80 (AOA) often play a key role in the nitrification process. Yokoe *et al.* (2015)
81 demonstrated a decrease in the copy number of *amoA* gene of AOB in soil by the
82 solarization and a subsequent recovery of the number by the quantitative PCR (qPCR)
83 method for the first time. This finding indicates that the qPCR estimation of the *amoA*
84 gene of AOB is possibly a quick and useful diagnosis of the decline and recovery of
85 ammonia-oxidizers over the MPN method that takes about one month to obtain the

86 viable counts. Recently, side effects of pesticides on ammonia-oxidizers have been
87 evaluated by qPCR determination of *amoA* transcripts and gene for AOB and AOA
88 (Feld *et al.* 2015). However, the change in AOB abundance estimated by the qPCR
89 method together with fluctuation in nitrifying activity by soil disinfections, not only
90 solarization but also fumigation or steam sterilization, has not yet been investigated so
91 far.

92 The objectives of the present study were to elucidate fluctuations in the
93 abundance of ammonia-oxidizers estimated by the qPCR and MPN methods and
94 dynamics of inorganic nitrogen (N) contents in the solarized soil and to show a
95 possibility of the molecular technique, the qPCR determination of *amoA* gene of
96 ammonia-oxidizers, for diagnosing suppression and restoration of nitrification following
97 soil solarization.

98

99 **2. Materials and methods**

100 ***2.1. Study site***

101 The greenhouse experiments with tomato (*Solanum lycopersicum* L.)
102 cultivation were conducted in a greenhouse at Miyazaki Agricultural Research Institute,
103 Miyazaki, Japan (32.004°N, 131.463°E) and in a greenhouse with isolated bed culture
104 in the Nagoya University Farm, Togo, Aichi, Japan (35.110°N, 137.082°E).

105

106 *2.1.1. Miyazaki Agricultural Research Institute (Miyazaki)*

107 The site was the same as that in the previous study (Murase *et al.* 2015), but in
108 a different greenhouse from the previous one. The soil was Typic Fluvaquent and the pH
109 (H_2O) was 6.0. Three plots with a randomized arrangement in triplicate (3.53 m^2 each)
110 were used: plots applied with liquid fertilizer prepared from distillery waste treatment of
111 barley shochu (1×10^4 kg ha^{-1} [fresh weight], N-P_2O_5-K_2O = 2.0-0.6-0.4 %; Japanese
112 liquor; Unkai Shuzo Co. Ltd., Miyazaki, Japan) before solarization (on 18 July, Sb) and
113 after solarization (on 29 August, Sa) and the control plot (NS) applied with the liquid
114 fertilizer (1×10^4 kg ha^{-1} [fresh weight]) on 29 August 2014. The solarization treatment
115 was conducted for 33 days from 18 July to 20 August as explained in detail by Murase
116 *et al.* (2015). The treatment with a laminated aluminum sheet to prevent elevation of
117 temperature was performed for the plot NS in the similar procedure and the plot NS was
118 designated as a non-solarized plot. Tomato seedlings were planted on 22 September.
119 Soil temperatures at 15 cm depth in the plots Sb and NS were measured every 10 min
120 from 6 to 20 August with a temperature data logger (Ondotori Jr. RTR-502, T&D
121 Corporation, Matsumoto, Japan).

122 Soil samples (about 500 g) were collected from the depth of 0–10 cm (at the
123 middle of tomato plant hills in the tomato cultivation period) using a trowel on 18 July
124 (before solarization [and before fertilization for the plot Sb]), 4 August (midpoint of the
125 solarization period), 20 August (termination of solarization), 19 September (about one

month after termination of solariztion) and 28 November (about 10 weeks after termination of solarization). The soil samples were sieved through a 2-mm mesh screen, kept at 4 ºC and subjected to the microbial and inorganic N analyses within a week. The soil samples for the DNA analysis were stored at –20 ºC until use.

2.1.2 Nagoya University Farm (NUF)

The greenhouse in the NUF was the same as that in the previous study and details of the bed culture, solarization and soil managements were already described (Yokoe *et al.* 2015). The soil was Hapludult and the pH (H_2O) was 5.9-7.4. Briefly, the greenhouse consisted of three beds (0.85 m width × 26 m length × 0.3 m depth; Kumiai Drain Bed, Katakura Chikkarin, Tokyo, Japan) with application (2×10^4 kg ha^{-1} [fresh weight]) of cattle manure compost (prepared in-house; bed A), compost of sewage sludge from food industry (N-P_2O_5-K_2O = 3.5-4.6-1.8 %, UT Compo, Kinuura Utility, Hekinan, Japan; bed B) and "bokashi" compost of animal and plant wastes (N-P_2O_5-K_2O = 4.0-5.5-3.0 %, Aging, Chlorella Industry, Tokyo, Japan; bed C). Tomato plants were cultivated twice a year from February to June and from August to December. The soil solarization has been performed in the greenhouse annually since 2004 and was conducted for 48 days from 18 June to 5 August in 2014. The soil was fertilized and plowed on 5 August and tomato seedlings were planted on 11 August. Soil temperatures at 7.5 and 15 cm (at the bottom of the bed) depths were measured hourly

146 throughout the solarization period at the center of the beds with a temperature data
147 logger (Ondotori Jr. TR-52i, T&D Corporation).

148 Soil samples were collected at three points in each bed (located about 6 m
149 apart) from the depths of 0–5, 5–10 and 10–15 cm (at the middle of tomato plant hills in
150 the tomato cultivation period) using three sampling cores (DIK-1801 100 mL stainless
151 steel sampling tube; Daiki Rika Kogyo, Tokyo, Japan) on 27 May (under cultivation of
152 tomato; only from bed B), 18 June (before solarization), 9 July (midpoint of the
153 solarization period), 5 August (termination of solarization [before fertilization]), 2
154 September (4 weeks after planting of tomato seedlings) and 21 October (11 weeks after
155 planting of tomato seedlings). The three soil samples from each depth were mixed for
156 each bed, sieved through a 2-mm mesh screen, kept at 4 ºC and subjected to the
157 microbial and inorganic N analyses within a week. The soil samples for the DNA
158 analysis were stored at –20 ºC until use.

159

160 ***2.2. DNA extraction from soil***

161 DNA for quantitative PCR was extracted from 0.5 g of soil samples using the
162 ISOIL for Beads Beating Kit for soil (NIPPON GENE, Tokyo, Japan) by the
163 bead-beating method according to the manufacture's instruction with two replicates.
164 The extracted DNA was dissolved in 100 μL of TE buffer (10mM Tris–HCl, 1mM
165 EDTA; pH 8.0).

166

167 ***2.3. Quantification of* amoA *of AOB and AOA***

168 *amoA* genes of AOB and AOA were quantified using the primer pairs
169 amoA1F/amoA2IR according to the method by Morimoto *et al*. (2011) as described by
170 Yokoe *et al.* (2015) and AOAamoA19IF/CrenamoA616r according to the method by
171 Tourna *et al.* (2008) and Morimoto *et al.* (2011) as described by Li *et al.* (2014),
172 respectively, with a Thermal Cycler Dice Real Time System (TaKaRa, Otsu, Japan).

173

174 ***2.4. Enumeration of viable number of ammonia-oxidizer***

175 The 10 g moist soil sample was dispersed in 90 mL of 10 mM Tris–HCl (pH
176 7.2) using a blender (HOMO BLENDOR, Sakuma, Tokyo, Japan) at the maximum
177 speed (16,000–18,000 rpm) for 3 min (Kanazawa *et al.* 1986) and a 10-fold dilution
178 series was prepared with the same buffer. A quantity of 0.5 mL of a 10^{-2}- to 10^{-8}-fold
179 diluted suspension with five replicates per dilution was inoculated to 1.5 mL of the
180 medium for enumeration of ammonia-oxidizers (Kimura 1992) in a 24-well
181 microtitration plate (Wada *et al.* 2008) and the plate was incubated at 30 ºC for 4 w
182 under dark conditions. Growth of ammonia-oxidizers was judged on the production of
183 nitrite and nitrate using Griess Ilosuvay reagent and zinc powder, respectively, and
184 viable numbers of ammonia-oxidizers were calculated from a table of MPNs for five
185 replicates per dilution at a dilution ratio of 10 according to Kimura (1992).

186

187 ***2.5. Inorganic nitrogen content in soil***

188 The 10 g moist soil was suspended in 90 mL of 2 M KCl solution for 1 h by
189 shaking and the suspension was filtered with a filter paper (Advantec no.6, Toyo Roshi,
190 Tokyo, Japan) for extraction of ammonium-N. The concentration of ammonium-N in the
191 filtrate was determined by the indophenol blue method using a UV spectrophotometer
192 (UV-2450, Shimadzu, Kyoto, Japan) (Hidaka 1997). For extraction of nitrite and nitrate,
193 the 10 g moist soil was suspended in 50 mL of distilled water for 1 h by shaking
194 (Kamewada 1997) and the suspension was filtered with a filter paper (Advantec no. 6)
195 and then a membrane filter (pore size 0.45 μm; Advantec Dismic-25CS). The
196 concentrations of nitrite- and nitrate-N were measured with an ion chromatograph
197 (PIA-1000, Shimadzu) as described by Baba *et al.* (2016). Extraction was conducted
198 each for triplicate soil samples from Miyazaki and it was duplicated for each soil
199 sample from NUF. Proportion of nitrate to inorganic N content was calculated.

200

201 ***2.6. Statistical analysis***

202 ANOVA, Tukey test, paired t-test, Friedman test and correlation analysis were
203 performed using the PAST program (Hammer *et al.* 2001). Logarithmically transformed
204 values of the copy numbers of *amoA* gene and viable numbers of ammonia-oxidizers
205 were used for the tests.

206

207 **3. Results**

208 ***3.1. Elevation of temperature by solarization***

209 Maximum soil temperatures above 40 ºC were recorded in 14 to 41 days in
210 the plot Sb of Miyazaki at 15 cm depth and the beds of NUF at 7.5 and 15 cm depths.
211 The temperature was significantly higher at 7.5 cm depth than at 15 cm depth in the
212 beds of NUF ($P < 0.001$, paired t-test). In the plot NS of Miyazaki, the temperature
213 ranged between 35 and 40 ºC in most days and the maximum temperatures above 40 ºC
214 were recorded only in 5 days (Fig. S1).

215

216 ***3.2. Fluctuation in abundance of ammonia-oxidizer***

217 *3.2.1. Copy number of bacterial* amoA *gene*

218 Copy number of ammonia-oxidizing bacterial *amoA* gene was conspicuously
219 decreased to one tenth or less of that before the solarization, even at the depth of 10–15
220 cm in the beds of NUF, by the solarization treatment (Figs. 1 and 2). A significant
221 difference ($P < 0.01$ or $P < 0.001$) was found among the treatments or the sampling
222 periods for the data in Miyazaki (Table S1). Among the treatments, the plots Sa and Sb
223 showed significantly lower copy numbers than the plot NS on 4 and 20 August and 19
224 September ($P < 0.01$, Tukey test). The copy number was significantly lower at the
225 termination of solarization on 20 August than the number before the solarization on 18

226 July in the plots Sb and Sa ($P < 0.01$, paired t-test) and the number in the tomato
227 cultivation period after the solarization on 28 November in the plot Sa ($P < 0.05$, paired
228 t-test). A significant difference ($P < 0.01$ or $P < 0.001$) was found for the data in each
229 bed of NUF among the sampling periods (Table S1). The copy number was significantly
230 lower at the termination of solarization on 5 August than the number before solarization
231 on 18 June ($P < 0.01$, paired t-test) and the number in the tomato cultivation period after
232 solarization on 21 October ($P < 0.001$ [bed A] or $P < 0.05$ [beds B and C], paired t-test).
233 In contrast, the decrease during the solarization in the plot NS of Miyazaki was not
234 significant ($P = 0.06$, paired t-test) and within one order of magnitude from 3.1×10^8
235 (18 July) to 3.5×10^7 (20 August) copies g^{-1} soil, though the number on 20 August was
236 significantly lower than the number on 28 November ($P < 0.01$, paired t-test).
237
238 *3.2.2. Copy number of archaeal* amoA *gene*
239 The decrease in the copy number of *amoA* gene of AOA was less evident than
240 that of AOB. The decrease was within one order of magnitude at most at the depth of 0–
241 5 cm in the beds A and B of NUF and the plots Sb and Sa of Miyazaki (Figs. 1 and 2).
242 The copy number of *amoA* gene of AOA in the beds A and B of NUF was not
243 significantly different among sampling dates ($P > 0.05$; Table S1). A significant
244 difference was found among sampling dates in the plots of Miyazaki ($P < 0.001$) and the
245 bed C of NUF ($P < 0.05$) (Table S1). The copy number in the bed C of NUF showed a

246 different trend (Fig. 2). The decrease in the copy number of *amoA* gene of AOA was
247 almost same as the decrease in the copy number of *amoA* gene of AOB from 18 June
248 (before the solarization) to 9 July (midpoint of the solarization period). The decrease in
249 the number from 18 June to 9 July in the bed C was not significant ($P = 0.07$, paired
250 t-test). The copy number of *amoA* gene of AOA at the termination of solarization on 20
251 August (Miyazaki) or 5 August (NUF) was not significantly different from the numbers
252 before the solarization on 18 July (Miyazaki) or 18 June (NUF) and in the tomato
253 cultivation period after the solarization on 28 November (Miyazaki) or 21 October
254 (NUF), respectively ($P > 0.05$, paired t-test). An exception was that the number in the
255 plot NS of Miyazaki on 28 November was slightly higher than the number on 20 August
256 ($P < 0.05$, paired t-test).
257
258 *3.2.3. Viable number of ammonia-oxidizer (MPN)*
259 Viable number of ammonia-oxidizers (MPN) was greatly decreased by the
260 solarization. The number was decreased to below the detection limit (about 36 cells per
261 gram of moist soil) of the MPN method on 4 August (midpoint of the solarization
262 period) in the plots Sb and Sa of Miyazaki and on 5 August (termination of the
263 solarization) at 0–5 cm and 5–10 cm depths in the beds of NUF (Figs. 1 and 2). The
264 number was significantly different among the treatments in Miyazaki ($P < 0.001$; Table
265 S1) and was lower in the plots Sa and Sb than in the plot NS on 4 and 20 August and 19

September ($P < 0.01$ or $P < 0.001$, Tukey test). The numbers were significantly different among the sampling dates ($P < 0.01$ or $P < 0.001$; Table S1). The number at the termination of solarization on 20 August (Miyazaki) or 5 August (NUF) was significantly lower than the number before the solarization on 18 July (Miyazaki; $P < 0.01$ [plot Sa] or $P < 0.05$ [plots Sb and NS], paired t-test) or 18 June (NUF; $P < 0.01$ [bed B] or $P < 0.05$ [beds A and C], paired t-test). The number in the tomato cultivation period after the solarization on 28 November (Miyazaki) or 21 October (NUF) was higher than the number on 20 August (Miyazaki; $P < 0.01$ [plot NS] or $P < 0.05$ [plots Sb and Sa], paired t-test) or 5 August (NUF; $P < 0.01$ [bed B] or $P < 0.05$ [beds A and C], paired t-test), where the number in the plot NS at the midpoint of the solarization period on 4 August was on the level of 10^4 cells g^{-1} dry soil (Fig. 1).

3.3. Inorganic N content in soil

Content of ammonium-N in soil tended to be increased by the solarization and decreased after the solarization concomitant with increase in the content of nitrate in soil (Figs. 1 and 2), where the nitrite content was almost a trace or below the detection limit. However, the fluctuation in values of the ammonium- and nitrate-N contents in soil could be influenced by the chemical property and application time of organic fertilizers as well as the nitrifying activity of the soil. This may make it difficult to infer nitrifying activity of soil simply based on fluctuation in the values of ammonium-N or

286 nitrate-N content. Therefore, proportion of nitrate to inorganic N content in soil (Kato *et*
287 *al.* 1984) instead of the absolute quantities of ammonium-N or nitrate-N was used as an
288 alternative indicator of the nitrifying activity. The proportion values in the plots Sb and
289 Sa of Miyazaki and in the beds A, B and C at 0–5 cm soil depth of NUF were
290 significantly different among the sampling dates ($P < 0.05$, Friedman test) and the
291 values are shown in Fig. 3. The proportion value tended to decrease by the solarization
292 and increase during tomato cultivation periods after the solarization. The values that
293 showed the lowest among the sampling dates, on 4 August (plot Sa; midpoint of the
294 solarization period) and 19 September (plot Sb; one month after termination of
295 solariztion), in Miyazaki (Fig. 3a) were significantly lower than the values before the
296 solarization on 18 July, respectively ($P < 0.05$ based on the studentized range). The
297 difference between 4 August and 28 November (10 weeks after termination of
298 solarization) in the plot Sa was also significant ($P < 0.05$ based on the studentized
299 range). In the beds of NUF at 0–5 cm soil depth, the lowest value was observed at the
300 midpoint of the solarization period on 9 July (Fig. 3b) and was significantly lower than
301 the values in the tomato cultivation period after the solarization on 21 October ($P < 0.05$
302 based on the studentized range).
303

304 **4. Discussion**

305 Maximum temperatures above 40 ºC were achieved for 2 weeks in all the

306 plots of Miyazaki and the beds of NUF except for the plot NS of Miyazaki (Fig. S1).
307 Fukui *et al.* (1981) reported that 14 days with the maximum temperature above 40 ºC
308 were needed to disinfect *Fusarium oxysporum* f. sp. *fragariae* and the lethal condition
309 was generally applicable to most of other soil-borne pathogens. Therefore, the
310 solarization treatment in the present experiments seemed to be almost sufficient for
311 controlling soil-borne diseases. The temperature condition ranging between 35 and 40
312 ºC except for 5 days in the plot NS was not lethal enough to disinfect soil-borne
313 pathogens. Although the plot NS was the control plot with the treatment for preventing
314 temperature elevation by solarization, the moderate elevation of temperature may have
315 slightly affected nitrification in the soil.

316 Viable number of ammonia-oxidizers (MPN) was greatly decreased by the
317 solarization and the decrease to below the detection limit of the MPN method was
318 observed in the plots of Miyazaki and the beds of NUF except for the plot NS and at the
319 depth of 10–15 cm of NUF (Figs. 1 and 2). Many investigations have shown the
320 detrimental effect of soil disinfestation on ammonia-oxidizers by the MPN method.
321 Nishihara (2008) and Wada *et al.* (2008) also reported the influences on the viable
322 counts of ammonia-oxidizers with the decrease to less than 1/100 to 1/1000 by
323 solarization treatments. The present study confirmed the findings in the previous studies
324 that solarization decreased viable number of ammonia-oxidizers. The number in the plot
325 NS was also decreased about three orders of magnitude (Fig. 1). The elevation of

326 temperature up to around 40 ºC (max. 41.7 ºC) in the NS plot, although it was a control
327 plot, may have affected the viable number of ammonia-oxidizers because Myers (1975)
328 reported that nitrification rate in soil declined rapidly at the incubation temperature over
329 35 ºC.

330 Copy number of *amoA* gene of AOB was decreased to less than 1/10 to 1/100
331 by the solarization in the plots of Miyazaki and the beds of NUF except for the plot NS
332 (Figs. 1 and 2). The decreasing trend of the copy number by the solarization in the beds
333 of NUF was almost same as that in the same field reported by Yokoe *et al*. (2015).
334 These findings confirmed that the great impact on AOB abundance by the solarization
335 was detected at the *amoA* gene level. On the other hand, the copy number also tended to
336 decrease in the plot NS of Miyazaki though the decrease was not significant ($P = 0.06$)
337 and within one order of magnitude, which was much smaller than the decreases in the
338 plots Sb and Sa. The soil temperature in the plot NS reached to around 40 ºC. This
339 elevation of temperature may have slightly affected the AOB abundance based on *amoA*
340 gene analysis since Xu *et al*. (2017) evaluated the growth of AOB in the incubation of
341 an acidic soil based on quantification of the *amoA* gene and found that it was strongly
342 inhibited at 40 ºC.

343 The decrease in the copy number of *amoA* gene of AOA was less than that of
344 AOB (Figs. 1 and 2), indicating that AOA exhibited somewhat thermotolerant traits.
345 Among seven AOA strains/enrichment cultures isolated from soil environments so far

346 (Lehtovirta-Morley *et al.*, 2016), the maximum growth temperature was 45 ºC
347 ('*Candidatus* Nitorosocosmicus franklandus' C13) (Lehtovirta-Morley *et al.*, 2016) to
348 47 ºC (*Nitrososphaera viennensis* EN76) (Tourna *et al.,* 2011). AOA might have a little
349 higher thermotolerance than AOB since typical AOB in soil have been generally
350 recognized as mesophiles (Hatzenpichler 2012). The AOA members inhabiting the soils
351 in the present study need to be examined for further investigation.

352 The decreasing and increasing trends in the proportion of nitrate to inorganic
353 N content in soil were observed in the periods of the solarization and tomato cultivation
354 after the treatment (Fig. 3). These findings indicate that nitrifying activity was
355 suppressed by the solarization and gradually recovered during the tomato cultivation
356 subsequent to the solarization. Accumulation of ammonium-N after solarization (Ihara
357 *et al.* 2014) and after soil disinfection treatments such as steam sterilization (e.g.,
358 Morikuni *et al.* 1999; Tanaka *et al.* 2003) has been reported in several studies and the
359 accumulation kept till 4 months (Tanaka *et al.* 2003). The proportion value in the plot
360 Sb of Miyazaki after 3 months of the solarization treatment was not fully recovered to
361 the same degree before the treatment (Fig. 3a). Recovery of the abundance of AOB was
362 a little retarded in the plot Sb compared with the plot Sa (Fig. 1) and this may have been
363 related to the increasing trend of the proportion value in the plot. In addition, the bed C
364 of NUF showed lower proportion values than those in the beds A and B (Fig. 3b). Some
365 properties of organic fertilizers, i.e. the liquid fertilizer prepared from distillery waste

366 treatment in the plots of Miyazaki and the "bokashi" compost in the bed C of NUF,
367 might have affected nitrification in the soils.

368 A positive correlation was found between the proportions of nitrate in
369 inorganic N content and the viable number of ammonia-oxidizers estimated by the MPN
370 method in the plots of Miyazaki and the beds of NUF at the depth of 0–5 cm (Fig. S2; r
371 = 0.663, $P < 0.001$). In addition, copy numbers of *amoA* gene of AOB and AOA
372 exhibited positive correlations with the proportion (Fig. S2; r = 0.468 [$P < 0.01$] and
373 0.610 [$P < 0.001$], respectively). These findings indicate that not only the viable number
374 of ammonia-oxidizers (MPN) but also the copy numbers of *amoA* gene could be a
375 potential indicator of nitrifying activity of the soils though the gene copy number would
376 represent the abundance of existent community of ammonia-oxidizers, not the
377 abundance of the active community. Relationships between the copy numbers of *amoA*
378 gene of AOB and AOA and the viable numbers of ammonia-oxidizers by the MPN
379 method in all the plots of Miyazaki and the beds of NUF are shown in Fig. 4. Both the
380 copy numbers showed positive correlations with the viable number ($P < 0.001$) and the
381 correlation coefficient for AOB (r = 0.838) was significantly higher than that for AOA (r
382 = 0.534) ($P < 0.001$). Contribution of AOA to ammonia oxidation in soil is still under
383 debate. The preference of AOA for low pH and low ammonium concentrations has been
384 hypothesized (Hatzenpichler 2012). The soil conditions with relatively high contents of
385 ammonium-N (Figs. 1 and 2) and near neutral pH values (5.9–7.4, Murase *et al.* 2015;

386 Yokoe *et al.* 2015) would have excluded major contribution of AOA to ammonia
387 oxidation of the soils in the present study. Small fluctuation in the copy number of
388 *amoA* gene except for the bed C of NUF (Fig. 1) might have been attributed to the
389 possible mixotrophic grwoth of AOA (Hatzenpichler 2012). In contrast, the copy
390 number of *amoA* gene of AOB was fluctuated concomitantly with the viable number of
391 ammonia-oxidizers (MPN) though range of the fluctuation in the copy number was less
392 than that in the viable number (Figs. 1, 2 and 4). Thus, the abundance of AOB based on
393 qPCR estimation of *amoA* gene is possibly a diagnostic indicator of suppression and
394 restoration of nitrification followed by solarization, which is an alternative to the
395 abundance of ammonia-oxidizers by the MPN method.

396 The present study revealed fluctuations in the abundance of
397 ammonia-oxidizers estimated by the qPCR and MPN methods and dynamics of
398 inorganic N contents in the solarized soil. The results showed a possibility of the
399 molecular technique, the qPCR determination of *amoA* gene of AOB, for diagnosing
400 suppression and restoration of nitrification following soil solarization. Because
401 enumeration of ammonia-oxidizers by the MPN method takes about one month to
402 obtain the viable counts, the qPCR estimation of the *amoA* gene of AOB is promisingly
403 a quick and useful diagnostic technique for evaluating the effect of solarization on
404 nitrification.

405

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511 **Figure legends**

512 Fig. 1. Abundance of copy numbers of *amoA* gene of ammonia-oxidizing bacteria and
513 archaea, viable number of ammonia-oxidizers and contents of ammonium- and
514 nitrate-nitrogen in solarized soils in the plots of Miyazaki. Open circles, open diamonds,
515 closed circles, open triangles and open squares indicate copy numbers of *amoA* of
516 ammonia-oxidizing bacteria, ammonia-oxidizing archaea, viable number of
517 ammonia-oxidizers, contents of ammonium- and nitrate-nitrogen, respectively. Bars
518 show standard errors of triplicate plots. Numbers marked with # are below the detection
519 limit of most probable number method (about 36 cells per gram of moist soil).

520

521 Fig. 2. Abundance of copy numbers of *amoA* gene of ammonia-oxidizing bacteria and
522 archaea, viable number of ammonia-oxidizers and contents of ammonium- and
523 nitrate-nitrogen in solarized soils in the beds of Nagoya University Farm. Open circles,
524 open diamonds, closed circles, open triangles and open squares indicate copy numbers
525 of *amoA* of ammonia-oxidizing bacteria, ammonia-oxidizing archaea, viable number of
526 ammonia-oxidizers, contents of ammonium- and nitrate-nitrogen, respectively. Numbers
527 marked with # are below the detection limit of most probable number method (about 36
528 cells per gram of moist soil).

529

530 Fig. 3. Proportion of nitrate in inorganic nitrogen content in solarized soils in the plots

531 of Miyazaki (a) and the beds at 0–5 cm soil depth of Nagoya University Farm (b).
532 Circles, triangles and squares denote plots Sb, Sa and NS (a) and beds A, B and C (b),
533 respectively. Bars show standard errors of triplicate plots of Miyazaki (a).
534
535 Fig. 4. Relationships between copy numbers of *amoA* gene of ammonia-oxidizing
536 bacteria (AOB) and ammonia-oxidizing archaea (AOA), and viable number of
537 ammonia-oxidizers (MPN).
538
539 Supplemental Fig. S1. Soil temperatures measured during solarization in the plots Sb
540 and NS at 15 cm depth of Miyazaki (a) and the beds A (b), B (c) and C (d) at 7.5 and 15
541 cm depths of Nagoya University Farm.
542
543 Supplemental Fig. S2. Relationships between proportion of nitrate in inorganic nitrogen
544 content and copy number of *amoA* gene of ammonia-oxidizing bacteria (AOB), viable
545 number (MPN) of ammonia-oxidizers and copy number of *amoA* gene of
546 ammonia-oxidizing archaea (AOA).
547

Fig. 1.

Plot Sb
Plot Sa
Plot NS
MPN (log cells g-1 dry soil)
amoA gene (log copies g-1 dry soil)
NH4+ or NO3- (mgN kg-1 dry soil)
Date
7/18
8/4
8/20
9/19
11/28
#

Fig. 2.

Bed A
Bed B
Bed C
0-5 cm
5-10 cm
10-15 cm
MPN (log cells g-1 dry soil)
amoA gene (log copies g-1 dry soil)
NH4+ or NO3- (mgN kg-1 dry soil)
Date
6/18 7/9 8/5 9/2 10/21
5/27 6/18 7/9 8/5 9/2 10/21
#

551

Fig. 3.

a
Miyazaki
b
NUF
$NO_3^-/NH_4^+ + NO_3^-$
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
7/18 8/4 8/20 9/19 11/28
6/18 7/9 8/5 9/2 10/21
Date
Date

552

Fig. 4.

MPN-AOB
MPN-AOA
r = 0.838***
r = 0.534***
MPN (log cells g-1 dry soil)
amoA gene (log copies g-1 dry soil)
1
2
3
4
5
6
7
8
5
6
7
8
9
10

553

Table S1. Variance analysis of logarithmically transformed copy numbers of *amoA* gene of ammonia-oxidizing bacteria (AOB) and archaea (AOA) and viable number of ammonia-oxidizers (MPN) in the plots of Miyazaki and the beds of Nagoya University Farm (NUF).

Site/Bed	Factor	d*f*	*amoA*		MPN
			AOB	AOA	
Miyazaki	Treatment (Sb, Sa and NS) (a)	2	**	ns	***
	Residue (plot)	6			
	Plot	8	***	ns	***
	Date (18 Jul, 4 Aug, 20 Aug, 19 Sep and 28 Nov) (b)	4	***	***	***
	a × b	8	***	ns	***
	Residue (plot) × b	24			
	Total	44			
NUF A	Soil depth (0–5, 5–10 and 10–15 cm) (a)	2	ns	ns	ns
	Date (18 Jun, 9 Jul, 5 Aug, 2 Sep and 21 Oct) (b)	4	**	ns	**
	Error (a × b)	8			
	Total	14			
NUF B	Soil depth (0–5, 5–10 and 10–15 cm) (a)	2	ns	ns	ns
	Date (27 May, 18 Jun, 9 Jul, 5 Aug, 2 Sep and 21 Oct) (b)	5	***	ns	***
	Error (a × b)	10			
	Total	17			
NUF C	Soil depth (0–5, 5–10 and 10–15 cm) (a)	2	ns	ns	ns
	Date (18 Jun, 9 Jul, 5 Aug, 2 Sep and 21 Oct) (b)	4	***	*	***
	Error (a × b)	8			
	Total	14			

*, ** and *** show a significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. ns indicates no significant difference.

Supplemental Fig. S1.

Supplemental Fig. S1. Soil temperatures measured during solarization in the plots Sb and NS at 15 cm depth of Miyazaki (a) and the beds A (b), B (c) and C (d) at 7.5 and 15 cm depths of Nagoya University Farm.

581

Supplemental Fig. S2.

Supplemental Fig. S2. Relationships between proportion of nitrate in inorganic nitrogen content and copy number of *amoA* gene of ammonia-oxidizing bacteria (AOB), viable number (MPN) of ammonia-oxidizers and copy number of *amoA* gene of ammonia-oxidizing archaea (AOA).