**Anaerobic Soil Disinfestation Impact on Soil Nutrients Dynamics and Nitrous Oxide Emissions in
Fresh-market Tomato**

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Abstract

Anaerobic soil disinfestation (ASD) is proposed as a pre-plant, non-chemical soil disinfestation technique to control several soilborne phytosanitary issues. Limited information is available on the impact of ASD on soil fertility, plant growth, and potential nutrient loss. The objectives of the current study were to evaluate the effects of ASD applied using composted poultry litter (CPL) and molasses as amendments, on soil redox potential, pH, temperature, soil nutrient content, plant biomass and nutrient accumulation, and nitrous oxide (N₂O) emissions. A field study was conducted on fresh-market tomato (*Solanum lycopersicum* L.) at two sites, Immokalee and Citra, FL, comparing ASD applied using a mix of CPL at the rate of 22 Mg ha⁻¹ and two rates of molasses [13.9 (ASD1) and 27.7 m³ ha⁻¹ (ASD2)] as a carbon-source to chemical soil fumigation (CSF). ASD treatment had a significant impact on soil redox potential, but did not affect soil pH or temperature. Soil treatment did not affect nitrous oxide emissions from intact polyethylene mulched beds at either location. Emissions ranged from 0 to 0.378 µg m⁻² h⁻¹ and from 8.8 to 39.8 µg m⁻² h⁻¹ in Immokalee and Citra, respectively. However, on day 21 after punching holes in the polyethylene film to transplant, N₂O emissions ranged from 1.56 to 4.83 and from 303.4 to 1480.1 µg m⁻² h⁻¹ in Immokalee and Citra, respectively. Emissions were higher in ASD than in CSF plots in Citra, but not in Immokalee. Molasses and CPL used in ASD treatments increased soil nutrients content, and particularly the availability of P and K. Results show no clear evidence of an increased risk of N loss with ASD compared to CSF. However, pre- and post-planting nutrient management should be adjusted to take into account the nutrients provided through the molasses and CLP application.

Keywords: biological soil disinfestation, molasses, composted poultry litter, nitrous oxide, phosphorous, potassium, chemical soil fumigation

1. Introduction

Anaerobic soil disinfestation (ASD) is a pre-plant non-chemical soil disinfestation treatment proposed as an alternative to chemical soil fumigation (CSF) for the control of several soil-borne diseases, plant-parasitic nematodes, and weeds in a variety of crops (Roskopf et al., 2015; Shennan et al., 2014; Shinmura et al., 1999). The basic principle of the technique, known also as ‘biological soil disinfestation’, is to create an anaerobic soil environment by incorporating readily decomposable carbon (C) rich organic amendments into the soil, which is saturated with water and covered with impermeable polyethylene film to prevent the diffusion of oxygen from the soil surface (Butler et al., 2014a; Shennan et al., 2014). The temporary shift of the soil environment, from aerobic to anaerobic conditions, that occurs for a few days, stimulates the growth of facultative and obligate anaerobic microorganisms, including *Clostridia* and *Bacilli* groups (Mowlick et al., 2012, 2013a, 2013b; van Agtmaal et al., 2015). Under anaerobic conditions, these microorganisms decompose the labile C, producing organic acids, aldehydes, alcohols, ammonia, and volatile organic compounds that are suppressive or toxic for several soil-borne pests and plant pathogens (Momma, 2008; Momma et al., 2006; Oka, 2010; van Agtmaal et al., 2015). While the exact mechanism of pest control has not been fully elucidated, the ASD technique proved to be effective against a number of soilborne pests (Butler et al., 2012a, 2012b; Lamers et al., 2010; Momma, 2008; Roskopf et al., 2015; Shennan et al., 2014). Besides soil pest control, the transient variation of soil redox potential, pH, and microbial populations caused by the ASD treatment (Momma, 2008; Momma et al., 2006), combined with amendment of the soil with organic matter, may have a significant impact on soil nutrient content and dynamics, plant nutritional status and growth, as well as on the loss of nutrients to the environment (Roskopf et al., 2015). In previous studies, ASD applied on different vegetable crops, increased soil nutrient content, improved plant growth, and provided higher fruit yield compared to the fumigated control (Butler et al. 2014a; Di Gioia et al., 2016). Nevertheless, information on the potential environmental impact of the ASD technique is inadequate and there are concerns about the potential risks of nutrient loss, especially during the treatment period prior to transplanting. It is possible that nutrients, particularly inorganic N, may be immobilized into the soil microbial biomass or may be subject to losses,

principally through leaching out of the root zone (Benoit et al., 2015). As anaerobic conditions are created during the ASD treatment, N losses may occur by denitrification with the reduction of nitrate (NO_3^-) to gaseous forms of N, including N_2 and N_2O , which is a potent greenhouse gas currently considered the most important substance contributing to stratospheric ozone depletion (Charles et al., 2017; Ravishankara et al., 2009).

A field study was conducted on fresh-market tomato grown in open-field in two locations in Florida to examine the potential implications of ASD for both the soil-plant system and the environment. The objectives were to evaluate the effects of ASD and the most common CSF practices on soil temperature, moisture, pH, redox potential, nutrient content, and soil N_2O emissions; to evaluate effects on plant growth and nutrient accumulation, and estimate the residual fertility and the potential risk of nutrient loss at the end of the crop season.

2. Materials and Methods

2.1. Experimental sites and treatments

Two field experiments were conducted on fresh-market tomato during the spring season of 2015 in southwestern (Immokalee) and north-central Florida (Citra). Conventional CSF was compared with two ASD treatments, which consisted of amending the soil with 22 Mg ha^{-1} of CPL and two rates of molasses [13.9 (ASD1) and 27.7 $\text{m}^3 \text{ha}^{-1}$ (ASD2)] as the C source. The first experiment was established on 2 Feb. 2015 at the University of Florida (UF) - Institute of Food and Agriculture Sciences – Southwest Florida Research and Education Center (SWFREC) in Immokalee, FL. The second experiment was established on 25 Mar. 2015, at the UF Plant Science Research and Education Unit (PSREU). Weather conditions and treatment effects on fruit yield and quality, weed and root-knot nematode control, and cumulative soil anaerobicity (mV hr) were reported previously by Di Gioia et al. (2016).

The three treatments, CSF, ASD1 and ASD2, were the same at both sites and were arranged in a randomized complete block design with four replications. The two sites differed in soil type. In Immokalee, the soil was a Spodosol classified as Immokalee fine sand (sandy, siliceous, hyperthermic

Arenic Haplaquods) and in Citra the soil was a Gainesville loamy sand (hyperthermic, coated typic
 quartzipsamments). In addition, there were minor differences in the way beds were established. In
 Immokalee, each of the four blocks consisted of one raised bed, 0.90 m wide, 0.20 m high and 60 m long
 and treatments were applied to 15-m long sections of the bed, leaving a 3-m space between plots. In Citra,
 each of the four blocks consisted of three beds 0.90 m wide and 15-m long. Prior to treatment application,
 the soil was rototilled and a starter fertilizer mix including N, P and potassium (K) was applied based on
 the specific requirement of each site. Nitrogen, P and K were applied at the rate of 34, 49 and 37 kg ha⁻¹ at
 the Immokalee site, and 56, 22 and 42 kg ha⁻¹ in Citra, respectively. The starter fertilizer mix was
 broadcast applied to the soil surface in 60 cm wide bands at both sites. Rounded false beds were formed
 by hilling the soil from a depth of 10 cm. ASD beds were amended with CPL at the rate of 22 Mg ha⁻¹ in
 combination with a 1:1 (v:v) water dilution of sugarcane molasses (Agricultural Carbon Source,
 TerraFeed, LLC, Plant City, FL, USA). Molasses had a density of 1,420 kg m⁻³, 22% water content, and a
 pH of 4.9-5.2. The molasses-water mix was applied to ASD1 and ASD2 plots at the rate of 27.7 and 55.4
 m³ ha⁻¹. [Table 1](#) shows the mean macro- and micro-nutrient content of both CPL and molasses, and the
 total amounts of nutrients incorporated in the soil with the ASD1 and ASD2 treatment application.
 After CPL and molasses application, the soil was tilled to a depth of 15 cm with a rotary cultivator, beds
 were formed and covered with a 0.03-mm black/white VaporSafe® TIF (Raven Industries Inc., Sioux
 Falls, SD, USA) polyethylene mulch containing an ethylene vinyl alcohol (EVOH) barrier layer.
 Simultaneously, two drip irrigation lines [20 cm emitter spacing, 0.98 L h⁻¹ emitter rate (Jain Irrigation
 Inc., Haines City, FL, USA)] were installed under the mulch in each bed, approximately 2.54 cm below
 the soil surface and 20 cm apart from the center of the bed. ASD plots were then irrigated with 5 cm of
 water (based on raised-bed area only) to saturate air-filled pore space in the top 10 cm of the bed and
 enhance the development of anaerobic conditions ([Butler et al., 2012](#)).
 On the same day, the control plots were fumigated by shank injection. The fumigant applied at each
 location was the most commonly used product for vegetable production in that region. In Immokalee,
 fumigation was with Pic-Clor 60 (Soil Chemical Corporation, Hollister, CA, USA) containing a mixture

of 1,3-dichloropropene (39.0%) and chloropicrin (59.6%) at the rate of 224 kg ha⁻¹. In Citra, the fumigant was Paladin™ (Arkema Inc., King of Prussia, PA, USA) composed of dimethyl-disulfide (DMDS, 79%) and chloropicrin (21%) at the rate of 496 L ha⁻¹. Fumigated plots were mulched immediately following CSF injection at both sites using the same material as for the ASD plots.

2.2. Crop transplanting and growing conditions

Tomato varieties were selected based on regional grower preferences. The varieties were cv. ‘Skyway 687’ (Enza Zaden, Salinas, CA, USA) in Immokalee and cv. ‘Tribute’ (Sakata, Morgan Hill, CA, USA) in Citra. Both are large, round fresh-market commercial tomato varieties. They were transplanted at the third true leaf stage, three weeks after treatment application, 24 Feb. and 17 Apr. 2015 in Immokalee and Citra, respectively. Tomatoes were transplanted in single rows at a distance of 0.45 m within the row and 1.8 m between rows, establishing a density of 12,000 plants ha⁻¹ at each site. Each of the twelve plots in the experiment contained 34 plants. Plants were trellised using the stake and weave method at both sites. Irrigation and fertigation differed by site. A hybrid seepage-drip irrigation system was used in Immokalee, while in Citra, the crop was watered by drip irrigation as described by [Di Gioia et al. \(2016\)](#). Fertigation started three weeks after planting (WAP) following UF/IFAS fertilizer recommendations (FDACS, 2005). At the Immokalee site, N and K were applied twice a week by fertigation using potassium nitrate (13-0-44) and ammonium nitrate (34-0-0). In Citra, fertilizer was applied by fertigation once per week using a 6-0-8 plus micro blend by Mayo Fertilizer Inc. (Mayo, FL, USA). Total season fertilizer rates applied by fertigation were 180 and 268 kg ha⁻¹ of N and 263 and 265 kg ha⁻¹ of K in Immokalee and Citra, respectively. Total season N, P and K fertilizer rates were 214, 49 and 300 kg ha⁻¹ in Immokalee, and 323, 22 and 307 kg ha⁻¹ in Citra, respectively. The quantity of nutrients applied with the starter fertilizer mix and by fertigation was not adjusted between treatments considering the amount of nutrient applied with the organic amendment in the ASD plots ([Table 1](#)), because it was assumed that only a portion of the latter would be available to the plants during the crop cycle. Moreover, given the potential effects of the temporary change of the soil redox potential and pH on the soil microbiome and microbial activity, limited information is available to estimate the potential mineralization rates of the organic material

incorporated in the soil in ASD plots. In addition, many growers will not abandon their standard practice of a broadcast application of slow-release fertilizer when adopting ASD, therefore this approach allowed an assessment of the “worst case scenario” for nutrient loss (Ozores-Hampton et al., 2015).

2.3. Soil temperature, moisture and redox potential

Soil temperature and volumetric water content were monitored during the three-week treatment period. Temperature sensors and soil moisture probes were installed before initial irrigation at both sites. Two 12-Bit temperature smart sensors (S-TMB-M006, Onset Computer Corporation, Pocasset, MA, USA) were installed at 5 and at 10 cm depths and two ECH2O® Dielectric Aquameter probes (EC-5, Decagon Devices Inc., Pullman, WA, USA) were installed at 10 cm depth in each plot. Both soil temperature and soil moisture probes were connected to an automatic data logging system (Onset Computer Corporation, Pocasset, MA, USA). Two oxidation-reduction potential sensors (Pt combination electrodes, Ag/AgCl reference, Sensorex, Garden Grove, CA, USA) were also installed before initial irrigation at 15 cm depth. These sensors were used to evaluate the occurrence of anaerobic soil conditions (indicated by redox potential; Eh) and to monitor the degree of anaerobiosis achieved in the soil during the first three weeks after treatment application. Electrodes were continuously monitored using an automatic data logging system (CR-1000 with AM 16/32 multiplexers, Campbell Scientific, Logan, UT, USA).

2.4. Surface air sampling and N₂O analysis

Nitrous oxide gas emissions were monitored from the top of the sealed, mulched beds 1, 2, and 3 or 4 days after treatment application, and at transplanting (21 days after treatment application), while punching holes in the polyethylene bed mulch. Emissions were collected using custom made static chambers made from 4.7 L stainless steel bowls. The bowls were placed on top of the polyethylene mulched bed in randomly selected positions and were sealed with grease. Each stainless steel bowl had a hole at the top of the head space on which was mounted a 9.5 mm silicone/PTFE septum (Alltech Microsep F-174N) by means of a stainless steel bulkhead union sealed with epoxy cement. Gas samples were collected at intervals of 15 minutes for 60 minutes at each monitoring time. Through the silicon septum of each static chamber, 10 mL of surface air were withdrawn using a gas tight plastic syringe attached to a 10-cm long

metal needle by means of a 3-4 cm Masterflex silicone rubber tube. Air samples collected were trapped in non-coated 10 mL Vacutainer® sampling tubes (BD Diagnostics, Franklin Lakes, NJ, USA). Tubes were stored in a cooler with ice immediately after taking the sample and then transferred to a freezer (-20 °C) in the laboratory until processing for analysis.

Nitrous oxide concentrations in the samples were measured by gas chromatography (GC) using a Perkin-Elmer Clarus 400 GC equipped with an electron capture detector (ECD) and a 80/100 Porapak Q stainless steel column measuring 1.8 m (length) × 3.18 mm (OD) × 2.1 mm (ID) (Supelco catalog # 12437). The carrier gas was UHP helium held at 10 psi. Samples were manually injected using a 250 or 500 µL Pressure-Lok gas syringe (Dynatech Precision Sampling Corp, Baton Rouge, LA, USA) into an unpacked injector held at 60 °C. The GC was operated in split/splitless mode where the split vent was open at 0.3 minutes and closed at 4.0 minutes. The GC oven was maintained at 35 °C and the ECD at 350 °C.

Calibration used external standard concentrations ranging from 2.1 to 42.9 µMol N₂O. Standard concentrations were made by injection of appropriate volumes of a N₂O standard gas (9.98 ppm certified N₂O in Helium at 1.04 atm pressure; Air Liquide catalog # T445-221) into the GC. Temperature and pressure corrections were accounted for using the Ideal Gas Law and Dalton's Law for partial pressures. Under conditions stated above the minimum detection limit (MDL) was 0.0017 µMol N₂O at a 95% confidence level (Hubaux, 1970). A storage study using vacutainer vials with a known concentration of N₂O kept at 4 °C in a refrigerator revealed less than 5% degradation in 35 days. All samples in the storage study and field study were warmed to ambient temperatures prior to injection.

2.5. Soil and soil solution sampling and nutrient analyses

Prior to treatment application, six soil cores (1.75-cm internal diameter) were taken randomly from each plot, excluding plot borders, at both sites at 30-cm depth. The six cores were combined in a single bulk sample for each plot. The same procedures were used for soil sampling at three weeks after treatment application (right before planting) and at the end of the crop season, 92 and 95 days after planting in Immokalee and Citra, respectively. Soil pH was measured directly in each moist soil sample using a FieldScout SoilStik pH meter (Spectrum Technologies, Inc, Aurora, IL, USA). Soil samples were air-

dried and then sieved (<2 mm). Samples were analyzed to determine the concentration of the N, C, P, K, calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), boron (B), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo) and nickel (Ni). Total N and C content were determined by combustion (NC Soil Flash EA1112, CE Elantech Inc., Lakewood, NJ, USA). Dry soil samples were digested utilizing a closed-vessel microwave-assisted digestion (MARS Express, CEM Corp., Matthews, NC, USA) according to U.S. EPA method 3052 (USEPA, 1997). Digested products were then analyzed by inductively coupled plasma atomic emissions spectrometry (ICP-AES; iCAP 6500, Thermo Scientific, Waltham, MA, USA) to determine the concentrations of P, K, Ca, Mg, Fe, B, Cu, Zn, Mn, Na, Mo and Ni.

A soil solution suction sampler (model SSAT, Irrrometer Company Inc., Riverside, CA, USA) was installed to a depth of 30 cm in the center of each experimental plot prior to initial irrigation. Two subsamples of soil solution were collected from each suction sampler by applying vacuum (-70 kPa) for 24 hours at 7, 14 and 21 days after treatment application. Soil solution samples collected in 20 mL plastic vials were transported from the field in an ice chest and then stored in a freezer (-20 °C) pending chemical analyses. Soil solution samples were analyzed by inductively coupled plasma atomic emissions spectrometry (ICP-AES; iCAP 6500, Thermo Scientific, Waltham, MA, USA) to determine the concentrations of P, K, Ca, Mg, Fe, B, Cu, Zn, Mn, Na, Zn, and Ni. The soil solution NO_3^- concentration was determined by ion chromatography, using an ICS 1000 (Dionex Corporation, Sunnyvale, CA, USA) with a conductivity detector with an IonPack AG14 pre-column and an IonPac AS14 separation column operating at 1800 – 1900 psi. The detector and suppressor were set at 17 mS and 24 miliamps, respectively. The eluent consisted of 20 mL of 3.5 mmol L⁻¹ of sodium carbonate and 1.0 mmol L⁻¹ of sodium bicarbonate solution. A 25 µL sample loop was used, and 5 mL samples were injected. Quality control standards and distilled-deionized water method blanks were used to ensure that the ion chromatography system was operating properly.

2.6. Plant growth and nutrient accumulation analysis

Plant growth was measured at 57 and 91 days after planting in Immokalee and Citra, respectively. Two representative plants were sampled from each plot and the fresh weight of leaves, stems, and fruit were measured and recorded for each plant. Plant material was dried to constant weight in a forced-air oven at 65 °C to determine the dry weight (DW). Dried leaf, stem, and fruit samples were finely ground through a mill to pass through a 20-mesh screen and 0.5 g of the dried plant tissues were analyzed to determine content of N, P, K, Ca, Mg, Fe, B, Cu, Zn, Mn, Na, Zn, and Ni. Total N content was determined by combustion (NC Soil Flash EA1112, CE Elantech Inc., Lakewood, NJ, USA). Dry plant samples were digested utilizing a closed-vessel microwave-assisted digestion (MARS Express, CEM Corp., Matthews, North Carolina, USA) according to U.S. EPA method 3052 (USEPA 1997). Digested products were then analyzed by inductively coupled plasma atomic emissions spectrometry (ICP-AES; iCAP 6500, Thermo Scientific, Waltham, MA, USA) to determine the concentrations of P, K, Ca, Mg, Fe, B, Cu, Zn, Mn, Na, Zn, and Ni.

2.7. Statistical analysis

Direct comparison of results between the two sites was not an objective of this study and therefore data from the two locations were analyzed separately. Data were subject to analysis of variance (ANOVA) using the GLM procedure in SAS Version 9.4 software (SAS Institute, Cary, NC, USA) following the randomized complete block design. All means were separated using Duncan's multiple range test at $\alpha = 0.05$. Soil solution nutrient concentration and N₂O data were analyzed using a repeated measures design according to [Littell et al. \(1998\)](#).

3. Results

3.1. Soil temperature, moisture, pH, and redox potential

Treatment during the three-week treatment period did not influence soil temperature at either location, although overall lower soil temperatures prevailed at Immokalee than at Citra during the three weeks after treatment applications ([Fig. 1](#)). In Immokalee, mean daily soil temperatures were on average 19.9 and 20.2 °C at 5 and 10 cm depth, respectively. Daily minimum temperatures ranged from 8.8 to 18.9 °C at 5

cm depth and from 7.6 to 19.3 °C at 10 cm depth. Maximum soil temperatures ranged from 20.7 to 40.0 °C at 5 cm depth and from 21.0 to 39.2 °C at 10 cm depth. In Citra, mean daily soil temperature averaged 29.5 and 28.7 °C at 5 and 10 cm depth, respectively. Daily minimum temperatures extended from 17.9 to 28.0 °C at 5 cm depth and from 15.7 to 27.7 °C at 10 cm depth. Maximum soil temperature ranged from 29.3 to 45.5 °C at 5 cm and from 27.3 to 42.5 °C at 10 cm depth.

Given that only ASD plots received 5 cm of initial irrigation, the volumetric soil water content during the three weeks after treatment application was higher in ASD than CSF plots at both locations (Fig. 2). In Immokalee, the average soil water content was 0.21, 0.25, and 0.26 m³ m⁻³ in CSF, ASD1, and ASD2 plots, respectively. Similarly, in Citra, the average soil water content was 0.18, 0.29, and 0.33 m³ m⁻³ in CSF, ASD1, and ASD2 plots, respectively.

Soil pH was similar in both locations prior to treatment application, on average 6.5 and 6.0 in Immokalee and Citra, respectively. Soil disinfestation treatment did not influence soil pH at three weeks after treatment application and soil pH was on average 7.1 in both locations at that point. Nor did soil disinfestation treatments affect soil pH at the end of the crop cycle, which averaged 6.3 in Immokalee and 6.0 in Citra.

Di Gioia et al. (2016) reported the significant effect of treatments on the cumulative mV hr under anaerobic conditions at both sites. ASD plots had significantly higher cumulative mV hr under anaerobic conditions as compared to CSF plots that maintained aerobic conditions. The soil environment is considered anaerobic when the redox potential is approximately below +200 mV, although the critical redox potential is slightly influenced by soil pH. Response to treatment was similar at both sites (Fig. 3). In Immokalee, negative Eh values were recorded in both ASD1 and ASD2 plots 30 hours after treatment application and lasted for approximately 60 hours in ASD1 plots and 350 hours in ASD2 plots. The CSF plots remained aerobic during the entire three-week treatment period. In Citra, negative Eh values were also recorded in both ASD1 and ASD2 plots 15 hours after treatment application and lasted for approximately 130 hours in ASD1 plots and 465 hours in ASD2 plots. Slightly anaerobic conditions were

observed in CSF plots in Citra, but Eh values remained positive during the entire three-week treatment period.

3.2. Plant growth and nutrient accumulation

Soil disinfestation treatments had no significant effect on plant fresh and dry weight at either location, nor did treatments affect plant macro or micro nutrient partitioning (Table 2). However, soil treatments did have a significant effect on plant aboveground nutrient accumulation at both sites, with plants grown in ASD plots, whether at the ASD1 rate of 13.9 m³ ha⁻¹ molasses or at the ASD2 rate of 27.7 m³ ha⁻¹, exhibiting higher total accumulation of most nutrients than plants grown in CSF plots.

Differences between treatments were more prevalent at the Immokalee site (Table 3) than the Citra site (Table 4). In Immokalee, plants grown in both ASD1 and ASD2 plots had significantly higher accumulations of K, Ca, Mg and Fe than CSF plants and significantly lower accumulations of Zn. Plants from ASD2 plots exceeded both ASD1 and CSF plants in N, P and K accumulation as well, while both ASD1 and ASD2 plants had higher accumulation of Ca, Mg and Fe than CSF plants. Soil treatments did not affect total plant accumulation of Na, B, Cu, Na, Mo or Ni.

There were also significant differences in nutrient accumulation in stems, leaves, and fruits at the Immokalee site. Both ASD1 and ASD2 plants had higher accumulations of Na, Zn, and Mn in stems than CSF plants, but soil treatments had no effect on accumulation of other elements in plant stems.

Regardless of molasses rate, accumulation of N, K, Ca, Mg, and Fe in plant leaves was consistently higher in plants grown in both ASD1 and ASD2 plots than in plants grown in CSF plots. Conversely, leaves of CSF plants accumulated significantly greater amounts of Zn and Mo than either ASD1 or ASD2 plants. Fruit of ASD2 plants accumulated significantly higher amounts of N, P, K, Ca, Mg, Na, Fe, B, Zn, and Mo compared to fruit from both ASD1 and CSF plants.

Fewer differences in nutrient accumulation as an effect of treatment emerged at Citra (Table 4). Total plant nutrient accumulations were not influenced by soil disinfestation treatments, with the exception of Mn accumulation, which was nearly three times greater in plants grown in ASD2 plot than those grown in CSF plots. ASD2 plots had a significantly higher accumulation of Zn in stems than CSF plant, but the

accumulation of other elements did not differ significantly between treatments. Although differences were not significant for most elements, plants grown in ASD2 plots accumulated significantly greater amounts of N, P, and Mn in their leaves than CSF plants, and ASD1 plants also accumulated significantly more N than did CSF plants. There were no significant differences attributable to soil treatments in nutrient accumulation in fruit.

3.3. Surface air sampling and N₂O analysis

Average N₂O emissions from the polyethylene mulched beds ranged from 0 to 0.378 $\mu\text{g m}^{-2} \text{h}^{-1}$ in Immokalee and from 8.8 to 39.8 $\mu\text{g m}^{-2} \text{h}^{-1}$ in Citra during the initial three (Citra) and four (Immokalee) days after treatment application (Fig. 4). No significant differences in N₂O emissions were observed among CSF, ASD1 and ASD2-treated soil during this period at either location. In contrast, emissions were at least one order of magnitude higher at both sites at 21 days after treatment application. Average N₂O emissions at 21 days after treatment application ranged from 1.56 to 4.83 $\mu\text{g m}^{-2} \text{h}^{-1}$ at Immokalee and from 303.4 to 1480.1 $\mu\text{g m}^{-2} \text{h}^{-1}$ at Citra. At 21 days after treatment application, emissions from both ASD treatments were significantly higher than the CSF treatments at Citra, but not in Immokalee. No significant differences were observed between ASD1 and ASD2-treated soil at either site at 21 days after treatment application.

3.4. Soil and soil solution nutrient content

Treatment effects on levels of plant nutrients present in the soil show patterns similar to those observed in accumulation of nutrients in plants. More differences among treatments emerged at Immokalee than at Citra and ASD2 exhibited the highest value for many nutrients. Soil disinfestation treatments did not influence the soil C:N ratio, with one exception. In Immokalee, the C:N ratio was higher in CSF plots than in ASD plots at the end of the crop cycle, regardless of the molasses rate (Table 5). In Immokalee, at transplanting, three weeks after treatment application, the content of K, Na, Fe, Cu, Zn, Mo and Ni measured in the top 30 cm of soil was higher in ASD than CSF plots, but no differences were observed for the other nutrients assessed. Regardless of molasses rate, ASD plots had higher N, P, Zn and Mo than CSF plots at the end of the crop cycle. No differences were observed for the other nutrients analyzed. In

Citra, only soil K, Na, and Mo were higher in ASD than CSF plots at three weeks after treatment application, and soil Ca content was higher only in ASD2 compared to CSF plots. Soil treatments had no effect on soil nutrient content at the end of the crop cycle, except for Na, which was higher in ASD2 than ASD1 and CSF plots.

During the three-week treatment period in Immokalee ([Table 6](#)), the nutrient concentration of the soil solution extracted by suction lysimeters at 30 cm depth was significantly influenced by soil disinfection treatments for all nutrients except Mo and a significant interaction was observed between soil treatments and assessment dates for all nutrients except Fe, B and Mo. At 7 and 14 days after treatment application, the soil solution NO_3^- concentration was significantly lower in both ASD1 and ASD2 plots than in CSF plots. The NO_3^- concentration increased in CSF plots from 134.0 mg L^{-1} to 160.1 mg L^{-1} at 7 and 14 days after treatment application, respectively. At 21 days after treatment application, the NO_3^- concentration of the soil solution in ASD plots remained stable while it decreased to 20.1 mg L^{-1} in CSF plots. As a result, there were no significant differences due to soil treatment in NO_3^- concentration at 21 days after treatment application. The concentrations in solution of K, Ca, Mg, Na, Fe, Mn and Ni were consistently significantly higher in both ASD1 and ASD2 plots than in CSF plots at 7, 14 and 21 days after treatment application. There was more variation in the effect of treatments for other nutrients. The P concentration was significantly higher in ASD1 plots than either CSF or ASD2 plots at 7 days after treatment application, but by 14 days after treatment application, P concentration increased in both ASD1 and ASD2 plots and was significantly higher in both treatments than in CSF plots at 14 and 21 days after treatment application. The concentration of K in solution was consistently higher in ASD than CSF plots, but a significant decrease in K concentration was observed in ASD1 at 21 compared to 7 and 14 days after treatment application. The concentration of Ca and Mg remained stable in CSF plots and decreased over time in ASD plots. While Ca concentrations in the soil solution were significantly higher in ASD plots than CSF plots at 7, 14 and 21 days after treatment application, the concentration in both ASD1 and ASD2 decreased between 14 and 21 days after treatment application. Similarly, Mg concentrations were consistently higher in ASD than CSF plots, but decreases over time, particularly in ASD1 plots.

Variations in micronutrient concentrations in the soil solution were less consistent for both treatments and assessment dates. Soil solution Na concentration was highest in ASD plots, but decreased between 7 and 14 days after treatment application in ASD1 plots and decreased between 14 and 21 days after treatment application in ASD2 plots. At 7 days after treatment application the soil solution concentration of Fe, Zn, Mn, Mo, and Ni in ASD1 plots was higher compared to CSF plots. However, differences in soil solution concentration of these elements were relatively small and inconsistent at the end of the three-week treatment period. In Citra, the quantity of soil solution collected was inadequate to accurately assess the potential for nutrient concentration from any soil treatment.

4. Discussion

The soil amendment with CPL (composted poultry litter) and molasses combined with the application of TIF (totally impermeable film) mulch and saturation of the soil with water caused a rapid shift of the soil environment from aerobic to anaerobic conditions, as observed in previous studies ([Butler et al., 2014a](#); [Strauss and Kluepfel, 2015](#)). The persistence of anaerobic conditions increased with the molasses rate, but was different between the two locations, likely due to differences in soil type or perhaps to the slight differences in soil temperature and water content at the two locations ([Fig. 1 and 2](#)). In contrast with the findings of other authors ([Momma, 2008](#); [Momma et al., 2006](#); [Rosskopf et al., 2015](#)), soil pH did not decrease in ASD plots and was not influenced by soil disinfestation treatments at either location. Instead, soil pH increased slightly, became nearly neutral (7.1) after the three-week treatment period, and then returned to initial levels at the end of the crop cycle. Similar results were reported by [Butler et al. \(2014b\)](#) who observed a higher soil pH in ASD treatment than in a fallow control in the presence of moderate temperatures (15 – 24 °C). The authors suggested that soil pH probably increased due to the release of basic cations from added organic materials without the release of sufficient organic acids during anaerobic degradation of the added C-source to generate a decrease in soil pH as reported for other ASD studies ([Momma et al., 2006](#); [Rosskopf et al., 2015](#)). Alternatively, as observed by [Katyal \(1977\)](#) in three

different soil types, ASD may have caused only a temporary drop of the soil pH, which stabilized near neutrality after 21 days. However, in this study, the ASD treatment probably induced a soil pH decrease that was counterbalanced by the application of CPL, which is characterized by a high pH (Tiquia and Tam, 2002). This suggests that the type of soil amendment used can influence soil pH under ASD treatment.

Soil temperatures recorded in Citra were optimal for the efficacy of ASD treatment, while the moderate temperatures recorded earlier in the spring season in Immokalee could be considered sub-optimal. Temperature can play a significant role in the effectiveness of ASD treatments (Butler et al., 2014b; Ebihara and Uematsu, 2014), but temperatures at both locations in this study resulted in anaerobic conditions, indicating that C application was adequate under both temperature regimes. Soil disinfestation treatments had no influence on soil temperature.

As expected, soil disinfestation treatments and especially the soil amendment with CPL and molasses significantly influenced the total soil macro- and micro-nutrient content (Table 5). However, no differences were observed between molasses rates at three weeks after treatment application. This suggests that increases in soil K, Na, and microelement (Fe, Cu, Zn, Mo, and Ni) content in ASD plots were mainly a result of the application of CPL. In fact, except for C and K, the addition of nutrients with the application of molasses was substantially lower than with the CPL (Table 1). Despite the addition of CPL and two rates of molasses, total C and N and the C:N ratio were not influenced by the soil disinfestation treatments three weeks after treatment application (Table 2). The similar C:N ratios across treatments may be explained by the application of a soil amendment with both C and N, by the relatively low C:N ratio of CPL (8:1), and by the rapid mineralization of the labile organic matter. Under aerobic conditions, decomposition of the added organic matter led to enhanced production of CO_2 and NH_4^+ relative to the fumigation treatment (Butler et al., 2014a; Katyal, 1977).

Soil NH_4^+ can volatilize to ammonia (NH_3) and accumulate under the TIF mulch and in soil pores, or in the presence of aerobic conditions, NH_4^+ would be nitrified to NO_2^- and then to NO_3^- by aerobic nitrifying soil bacteria. However, when the soil environment shifts to anaerobic conditions, nitrification is limited.

As a result, the reverse process, dissimilatory nitrate reduction to ammonium (DNRA) performed by various anaerobic bacteria may become prevalent, along with the denitrification. The latter is operated mainly by facultative aerobic bacteria that can grow in the absence of oxygen reducing NO_3^- and NO_2^- to N_2O and N_2 (Roskopf et al., 2015; Tiedje, 1988). Moreover, NO_3^- present in the soil may be subject to loss by leaching during the three-week treatment period if significant rainfall events (76 mm of rain in 3 days or 102 mm in 7 days; Simonne and Hochmuth, 2011) occur.

In Immokalee, the higher concentration of P, K, Ca, and Mg measured in the soil solution of ASD plots compared to CSF plots indicates that ASD may substantially increase the availability of these nutrients in the soil. Considering that in this study, the main source of P and K were the CPL and the molasses, respectively, these results highlight the importance of taking into account in the crop fertilization plan, the amount of nutrients added to the soil with the organic amendments, in order to avoid nutrient excess and potential losses.

The NO_3^- concentration in the soil solution was lower in ASD than in CSF plots during the entire three-week treatment period. In addition to the $\text{NO}_3\text{-N}$ applied with the starter fertilizer, the high NO_3^- concentration observed in the soil solution of CSF plots could be derived from chloropicrin hydrolysis (Castro and Blesler, 1981) and in part from the mineralization of killed microorganisms resulting from the fumigation (Hansen et al., 1990). In ASD plots, considering the relatively high C:N ratio of the molasses (28:1) added to the soil and the rapid microbial population growth, it is possible that high amounts of N were immobilized by soil microorganisms. Alternatively, the low NO_3^- concentration of the soil solution observed in ASD plots over the three weeks after treatment application may have been due to the prevalence of $\text{NH}_4\text{-N}$ over $\text{NO}_3\text{-N}$ as observed by Butler et al. (2014a) in soil amended with CPL under moderate anaerobic conditions and by Katyal (1977) in flooded soil amended with organic matter. These results suggest that the anaerobic conditions created with the ASD treatment substantially limited the activity of the obligate aerobic bacteria responsible for the ammonium nitrification. The lower NO_3^- concentration in the soil solution of ASD plots could also be due to denitrification during the anaerobic phase (Roskopf et al., 2015; Tiedje, 1988).

Nitrous oxide is produced in soils through microbial nitrification and denitrification (Maag and Vinther, 1996). Denitrification is considered the major source of N₂O production in ASD plots since anaerobic conditions prevailed (Fig. 3). The key factor limiting denitrification in most mineral soils is the availability of an organic C-source (Charles et al., 2017; Cochran et al., 1997; Flessa and Beese, 1995). Incorporation of CPL and molasses into the ASD treatments added an estimated 9,828 and 14,589 kg ha⁻¹ of C to the ASD treated soil. This, combined with the initial addition of water, resulted in reductions in redox potentials within the systems that were conducive for denitrification (Fig. 3). Increased N₂O emissions from the ASD treatments at both locations 21 days after treatment application may have been due to favorable soil redox potentials (Charles et al., 2017). Yu et al. (2001) reported that N₂O accumulated significantly for all soils tested at redox potentials ranging between +120 and +250 mV. In their study, N₂O emissions were low when the redox potential values were higher than +250 mV or lower than +120 mV. Since those studies were performed using paddy soils in China and agricultural soils planted to maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) in Belgium, there is some uncertainty regarding anaerobic boundaries in soils from different regions of the world. In the current study, the redox potentials for the ASD treatments were between 0 and +250 mV from 16 d to 21 d at Citra; and 0 to +300 mV in Immokalee. Earlier in the ASD treatments, redox potentials were lower than +120 mV, suggesting that N₂O emissions were lower if these systems behave as described by Yu et al. (2001). Interestingly, redox potential for the CSF treatments were between +200 and +250 mV for Immokalee and between 0 and +200 mV for Citra throughout the 21 days after treatment application. The lack of significant N₂O emissions was probably due to C limitations. Nevertheless, in Immokalee, where the level of N₂O emissions in CSF plots was similar to that of ASD plots, it is possible that the chloropicrin used as the fumigant standard stimulated the production of N₂O as observed in previous studies (Spokas and Wang, 2003; Spokas et al., 2005, 2006). Aside from the availability of inorganic N and available C, other factors modulating microbial N₂O production may include soil temperature, soil moisture, and pH (Knowles, 1982; Luo et al., 2013; Schaufler et al., 2010; Wu et al., 2010). Soil temperature directly impacts microorganism activity, soil

aeration, substrate availability, and redistribution. Previous studies reported that N₂O emissions were synchronized with surface soil temperature (Bremner et al., 1980; Cheng et al., 2014; Schaufler et al., 2010). Lu and Xu (2014) also reported that temperature increases in peat soil under flooding increased N₂O emission rates and cumulative emissions significantly. It is likely that a portion of the emissions measured after polyethylene film punching on 21 days after treatment application was due to accumulation of N₂O under the TIF mulch during the three-week period prior to sampling. Although the 21 days after treatment application N₂O emission rates were higher from ASD-treated plots in Citra, rates were similar to those resulting from urea application in Chinese vegetable production (Xiong et al., 2006). Instead, the peak N₂O emission rates measured in Citra, 21 days after treatment application, were lower than those measured in Scotland, in a grassland system amended with poultry manure at rates that provided levels of total N similar to those applied in ASD2 plots combining CPL and molasses in this study (Jones et al., 2005).

An improved understanding of total N₂O emissions resulting from ASD and conventional fumigation systems could be gained by season-long assessments.

One other notable observation in this study is the difference in the magnitude of N₂O emissions between the two sites (Fig. 4). Average emissions at the Citra site were typically an order of magnitude higher than those at Immokalee. Soil temperature and moisture may have influenced these emissions. Maag and Vinther (1996) reported that both factors significantly influenced N₂O emissions from sandy loam soils, while only temperature influenced emissions from coarse sandy soils. The soil at the Citra site was a loamy sand, whereas the Immokalee soil was a fine sand. Furthermore, soil temperature (Fig. 1) and moisture (Fig. 3) for the ASD treatments tended to be higher at Citra than at Immokalee.

The soil treatments did not result in significantly different effects on tomato plant growth and plant biomass partitioning between leaf, stem, and fruit, either on fresh or dry weight basis. These results do not explain the higher fruit yield observed in the same study at Immokalee for plants grown on ASD soil (Di Gioia et al., 2016). Nevertheless, the higher fruit yield may be explained in part by the improved nutritional status of the ASD plants. The higher total plant macronutrient accumulation, especially in

ASD2 plants, was likely due to a higher availability of nutrients during the crop cycle in soil amended with CPL and molasses. These results corroborate the findings of [Butler et al. \(2014a\)](#) who observed higher concentrations of N, P, K, Ca and Mg in plants grown in solarized soil amended with CPL or CPL and molasses.

At the end of the crop cycle, the amounts of nutrients found in the top 30 cm of soil in both sites suggest that the ASD treatment may have a residual fertility effect after the first crop cycle. In this case, residual nutrients may be exploited in a double cropping system ([Butler et al., 2014a](#)) or need to be properly managed (i.e. introducing suitable cover crops as catch crops) to minimize the risk of nutrient loss into the environment. Alternatively, soil should be amended with lower rates of CPL or with organic sources characterized by a lower nutrient content. Moreover, pre-plant fertilization should be avoided or reduced and in-season fertigation needs to be adjusted, taking into consideration the amount of nutrients added to the soil with the organic amendment.

5. Conclusions

In conclusion, the results of this study suggest that the ASD treatment using CPL and molasses has a significant impact on soil fertility, increasing the soil nutrient content and improving crop nutrition and nutrient accumulation. Nevertheless, nutrients added to the soil with the organic amendments, and particularly P and K should be adequately accounted to avoid surplus of nutrients and potential losses, especially during the three-week treatment period prior to crop establishment. The data presented here are preliminary in this regard and additional testing is required. Given the residual fertility of the organic amendment, additional losses of nutrients may occur at the end of the crop cycle or may increase the potential for employing the system over multiple cropping cycles. Moreover, the amendment of the soil with a C-source and the anaerobic conditions created during the ASD treatment may facilitate denitrification, thereby increasing N₂O emissions. Therefore, the application of the ASD technique on a larger scale will depend on a better understanding and management of the nutrients added to the soil with the incorporation of the organic amendments. Additional studies are required to further assess the

potential environmental impact of the ASD technique and evaluate the possibility of avoiding the application of pre-plant fertilizer or to use alternative organic materials characterized by lower nutrient concentrations without reducing the efficacy of ASD in managing soil-borne diseases, plant parasitic nematodes, and weeds.

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647 **Table 1.** Mean ($\pm$ standard deviation; n = 3) nutrient content of molasses and composted poultry litter and
648 total amounts of nutrients applied with the anaerobic soil disinfestation (ASD) treatments in Immokalee
649 and Citra, FL, in the spring of 2015.

Nutrient	Molasses		Composted poultry litter (CPL)		ASD1 ^z			ASD2 ^z		
	Mean	SD	Mean	SD	Molasses	CPL	Total	Molasses	CPL	Total
	g L ⁻¹		g kg ⁻¹		kg ha ⁻¹			kg ha ⁻¹		
N	12.3	$\pm$ 0.75	26.1	$\pm$ 1.46	171	574	745	341	574	915
C	342.5	$\pm$ 12.59	230.3	$\pm$ 25.72	4,748	5,067	9,815	9,489	5,067	14,556
P	0.8	$\pm$ 0.01	14	$\pm$ 0.82	11	308	319	22	308	330
K	51.6	$\pm$ 0.52	25	$\pm$ 0.57	715	550	1,265	1,430	550	1,980
Ca	9.8	$\pm$ 0.09	22.6	$\pm$ 0.46	136	497	633	272	497	769
Mg	3.8	$\pm$ 0.03	5.2	$\pm$ 0.02	53	114	167	105	114	220
Na	0.8	$\pm$ 0.01	8.6	$\pm$ 0.28	11	189	200	22	189	211
Fe	125.1	$\pm$ 2.12	396.6	$\pm$ 88.84	1.73	8.73	10.46	3.47	8.73	12.19
B	6.8	$\pm$ 0.88	48.8	$\pm$ 1.22	0.09	1.07	1.17	0.19	1.07	1.26
Cu	2.6	$\pm$ 0.14	421.2	$\pm$ 12.83	0.04	9.27	9.30	0.07	9.27	9.34
Zn	11.2	$\pm$ 0.2	478.1	$\pm$ 10.47	0.16	10.52	10.67	0.31	10.52	10.83
Mn	8.3	$\pm$ 0.37	418.8	$\pm$ 1.89	0.12	9.21	9.33	0.23	9.21	9.44
Ni	1.3	$\pm$ 0.34	9.8	$\pm$ 3.84	0.02	0.22	0.23	0.04	0.22	0.25
Mo	1.1	$\pm$ 0.02	4.3	$\pm$ 0.57	0.02	0.09	0.11	0.03	0.09	0.13

650

651 ^z ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses and 22 Mg ha⁻¹ of composted poultry
652 litter), ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses and 22 Mg ha⁻¹ of composted
653 poultry litter).

Table 2. Soil treatment effects on leaves, stem, fruit, and total plant fresh and dry weight of fresh-market tomatoes grown in Immokalee (University of Florida/Institute of Food and Agricultural Science/Southwest Florida Research and Education Center) and in Citra (University of Florida/Plant Science Research and Education Unit) in the spring of 2015. ^z

Soil treatment ^y	Fresh weight (g plant ⁻¹)				Dry weight (g plant ⁻¹)			
	Leaves	Stem	Fruit	Total	Leaves	Stem	Fruit	Total
Immokalee, FL								
CSD	668.6	419.6	1,860.9	2,949.1	70.6	43.3	48.6	162.5
ASD1	763.8	449.4	1,879.2	3,092.4	79.5	43.3	50.4	173.2
ASD2	774.1	462.8	1,695.4	2,932.3	81.7	44.2	45.4	171.3
<i>P value</i>	<i>0.71</i>	<i>0.91</i>	<i>0.79</i>	<i>0.92</i>	<i>0.67</i>	<i>0.99</i>	<i>0.80</i>	<i>0.91</i>
Citra, FL								
CSD	153.6	135.0	657.9	946.5	26.9	23.1	42.5	92.5
ASD1	296.1	210.4	837.8	1,344.3	47.0	35.1	52.3	134.4
ASD2	374.4	274.9	809.4	1,458.6	56.9	44.9	55.6	157.4
<i>P value</i>	<i>0.10</i>	<i>0.14</i>	<i>0.78</i>	<i>0.43</i>	<i>0.15</i>	<i>0.17</i>	<i>0.80</i>	<i>0.34</i>

^z Reported values are averages of four replications. Means followed by different letters within each column and location are significantly different at $\alpha = 0.05$ by Duncan's multiple range test. ^y CSF (chemical soil fumigation), ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses), ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses).

Table 3. Soil treatment effects on macro- and micro-nutrients partitioning and accumulation in 57-day-old tomato plants grown in Immokalee, during the spring of 2015.^z

Plant portion	Soil treatment	N	P	K	Ca	Mg			Na	Fe	B	Cu	Zn	Mn	Mo	Ni
		kg ha ⁻¹						g ha ⁻¹								
Stems	CSF	8.5	3.3	21.7	5.2	1.9			636 a	17.7	10.4	26.9	150.3 a	31.8 a	1.2	0.6
	ASD1	9.2	3.5	24.8	5.6	1.9			450 b	17.4	9.7	25.6	97.2 b	22.6 b	1.1	0.4
	ASD2	9.6	3.7	25.3	5.7	1.9			410 b	17.7	11.5	22.8	91.7 b	19.7 b	1.2	0.3
<i>P value</i>		0.30	0.15	0.06	0.49	0.93			0.01	0.99	0.15	0.32	0.0001	0.0001	0.55	0.45
Leaves	CSF	27.1 b	5.5	26.1 b	20.2 b	4.5 b			1,324	39.4 b	55.2	356.3	88.0 a	234.5 a	4.2 a	0.5
	ASD1	32.9 a	5.3	33.3 a	25.0 a	6.2 a			1,166	58.6 a	54.6	366.4	59.8 b	214.2 ab	3.1 b	0.6
	ASD2	34.4 a	5.8	34.7 a	25.0 a	6.0 a			1,118	51.1 a	50.1	338.4	57.3 b	187.7 b	3.2 b	0.5
<i>P value</i>		0.01	0.35	0.0001	0.0001	0.0001			0.45	0.004	0.61	0.70	0.003	0.03	0.03	0.53
Fruits	CSF	17.7 b	5.1 c	33.1 c	1.4 c	1.5 c			358 b	27.1 ab	14.1 b	13.8	36.6 b	16.3 a	1.1 b	0.3
	ASD1	15.6 c	5.6 b	39.9 b	1.7 b	1.7 b			342 b	26.2 b	12.6 c	13.1	35.0 b	13.4 b	1.0 b	0.7
	ASD2	20.1 a	6.5 a	47.1 a	2.0 a	2.0 a			406 a	29.6 a	16.2 a	14.5	41.6 a	14.2 b	1.2 a	0.9
<i>P value</i>		0.0001	0.0001	0.0001	0.0001	0.0001			0.03	0.04	0.001	0.27	0.0001	0.006	0.004	0.38
Total plant	CSF	53.3 b	13.8 b	80.8 c	26.8 b	7.9 b			2,318	84.3 b	79.7	397.0	274.9 a	282.6 a	6.5	1.4
	ASD1	57.7 b	14.4 b	97.9 b	32.3 a	9.8 a			1,958	102.2 a	76.9	405.1	192.1 b	250.3 ab	5.3	1.7
	ASD2	64.1 a	15.9 a	107.0 a	32.6 a	9.9 a			1,934	98.4 a	77.8	375.7	190.6 b	221.6 b	5.7	1.7
<i>P value</i>		0.006	0.01	0.0001	0.0003	0.0001			0.25	0.01	0.89	0.69	0.0001	0.01	0.08	0.79

^z Reported values are averages of four replications. Means followed by different letters within each column and plant portion are significantly different at $\alpha = 0.05$ by Duncan's multiple range test. ^y CSF (chemical soil fumigation), ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses), ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses).

Table 4. Soil treatment effects on macro- and micro-nutrients partitioning and accumulation in 91-day-old tomato plants grown in Citra, during the spring of 2015.^z

Plant portion	Soil treatment ^y	kg ha ⁻¹						g ha ⁻¹									
		N	P	K	Ca	Mg	Na	Fe	B	Cu	Zn	Mn	Mo	Ni			
Stems	CSF	4.4	0.6	6.1	4.0	0.8	113.5	12.2	4.2	8.5	4.3	b	8.2	0.6	0.2		
	ASD1	8.1	1.2	11.3	5.1	1.4	139.5	22.9	6.2	8.5	15.4	ab	13.1	1.0	0.4		
	ASD2	10.3	1.8	16.7	5.3	1.4	168.3	23.8	6.1	11.3	24.3	a	20.6	1.2	0.3		
<i>P value</i>		<i>0.07</i>	<i>0.06</i>	<i>0.07</i>	<i>0.58</i>	<i>0.15</i>	<i>0.40</i>	<i>0.40</i>	<i>0.37</i>	<i>0.17</i>	<i>0.04</i>		<i>0.07</i>	<i>0.14</i>	<i>0.36</i>		
Leaves	CSF	8.0	b	0.6	b	6.1	13.7	1.8	188.8	22.6	23.4	67.9	6.3	52.4	b	1.5	0.2
	ASD1	20.2	a	1.5	ab	15.3	23.2	3.4	295.5	47.2	41.9	93.5	15.8	98.6	ab	2.7	0.3
	ASD2	22.8	a	1.9	a	21.6	27.6	4.6	389.1	53.9	45.1	129.2	26.5	170.1	a	3.9	0.3
<i>P value</i>		<i>0.05</i>	<i>0.05</i>	<i>0.11</i>	<i>0.15</i>	<i>0.11</i>	<i>0.19</i>	<i>0.15</i>	<i>0.25</i>	<i>0.15</i>	<i>0.74</i>		<i>0.03</i>	<i>0.11</i>	<i>0.31</i>		
Fruits	CSF	13.5		1.9		18.2	1.1	0.9	169.8	17.3	4.8	4.9	11.1	7.2	1.5	0.2	
	ASD1	16.5		2.5		25.1	1.4	1.2	185.4	21.7	6.9	6.6	18.6	9.5	1.6	0.5	
	ASD2	17.6		2.8		28.4	1.2	1.2	208.8	23.9	7.3	7.0	20.9	10.9	1.8	0.3	
<i>P value</i>		<i>0.84</i>	<i>0.72</i>	<i>0.70</i>	<i>0.77</i>	<i>0.85</i>	<i>0.91</i>	<i>0.75</i>	<i>0.78</i>	<i>0.75</i>	<i>0.49</i>		<i>0.75</i>	<i>0.93</i>	<i>0.13</i>		
Total	CSF	25.9		3.0		30.5	18.7	3.4	472.1	52.1	32.3	81.3	21.8	67.7	b	3.6	0.5
	ASD1	44.8		5.3		51.7	29.8	5.9	620.5	91.9	55.0	108.5	49.9	121.2	ab	5.2	1.1
	ASD2	50.7		6.6		66.8	34.1	7.2	766.2	101.6	58.5	147.4	71.8	201.6	a	6.8	0.9
<i>P value</i>		<i>0.21</i>	<i>0.26</i>	<i>0.29</i>	<i>0.20</i>	<i>0.20</i>	<i>0.43</i>	<i>0.26</i>	<i>0.28</i>	<i>0.13</i>	<i>0.14</i>		<i>0.04</i>	<i>0.31</i>	<i>0.18</i>		

^z Reported values are averages of four replications. Means followed by different letters within each column and plant portion are significantly different at $\alpha = 0.05$ by Duncan's multiple range test. ^y CSF (chemical soil fumigation), ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses), ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses).

677 **Table 5.** Mean nutrient content of soil sampled to 30 cm-depth before treatment application, at planting (21 days after treatment application), and
678 at the end of the crop cycle, in Immokalee and in Citra in the spring of 2015.^z

Soil Treatment ^y	C:N ratio	N	C	P	K	Ca	Mg	Na	Fe	B	Cu	Zn	Mn	Mo	Ni
kg ha ⁻¹															
Immokalee, FL															
Pre-treatment															
Untreated	14.2	1,454	20,529	43.8	112	141	64.1	21.76	3.99	0.01	0.35	1.00	0.91	0.01	0.11
Planting															
CSF	14.1	1,908	26,201	55.3	68 b	158	47.1	26.2 b	3.65 b	0.45	0.38 b	0.75 b	1.89	0.03 b	0.09 b
ASD1	12.8	1,864	23,550	68.0	891 a	137	56.0	131.6 a	8.37 a	2.03	2.59 a	1.49 a	2.30	0.11 a	0.20 a
ASD2	14.3	1,992	27,075	73.4	1,172 a	197	77.5	119.4 a	8.60 a	1.51	2.28 a	1.52 a	2.98	0.11 a	0.31 a
<i>P-value</i>	<i>0.54</i>	<i>0.98</i>	<i>0.86</i>	<i>0.30</i>	<i>0.001</i>	<i>0.43</i>	<i>0.23</i>	<i>0.004</i>	<i>0.02</i>	<i>0.11</i>	<i>0.004</i>	<i>0.02</i>	<i>0.48</i>	<i>0.002</i>	<i>0.01</i>
End of the crop															
CSF	14.2 a	1,564 b	21,936	24.5 b	41	79.8	14.4	23.46	4.92	0.42	0.59	0.57 b	1.08	0.02 b	0.10
ASD1	13.1 b	2,139 a	27,892	46.0 a	54	77.9	23.5	53.74	5.56	0.58	0.91	0.74 a	0.81	0.03 a	0.16
ASD2	13.3 b	2,159 a	28,549	43.7 a	107	93.8	28.0	66.46	4.81	0.74	0.82	0.61 ab	0.70	0.04 a	0.13
<i>P-value</i>	<i>0.03</i>	<i>0.05</i>	<i>0.09</i>	<i>0.01</i>	<i>0.33</i>	<i>0.61</i>	<i>0.17</i>	<i>0.10</i>	<i>0.23</i>	<i>0.13</i>	<i>0.13</i>	<i>0.05</i>	<i>0.07</i>	<i>0.02</i>	<i>0.30</i>
Citra, FL															
Pre-treatment															
Untreated	18.6	2,470	45,263	21.0	64	33	5.7	7.78	20.42	0.16	0.22	0.10	0.35	0.02	0.10
Planting															
CSF	17.38	2,929	50,113	24.5	218 b	41 b	6.6	14.6 b	22.25	0.39	0.20	0.04	0.31	0.02 b	0.02
ASD1	16.96	3,315	52,375	35.9	1,869 a	176 ab	60.1	223.5 a	25.31	0.65	2.84	0.54	2.59	0.10 a	0.21
ASD2	15.27	4,085	56,050	40.7	2,178 a	276 a	104.6	203.5 a	29.46	1.17	2.47	0.67	4.76	0.08 a	0.26
<i>P-value</i>	<i>0.27</i>	<i>0.28</i>	<i>0.60</i>	<i>0.50</i>	<i>0.02</i>	<i>0.03</i>	<i>0.06</i>	<i>0.003</i>	<i>0.60</i>	<i>0.10</i>	<i>0.10</i>	<i>0.30</i>	<i>0.08</i>	<i>0.03</i>	<i>0.08</i>
End of the crop															
CSF	23.97	1,948	46,368	17.7	103	102	13.1	13.4 b	23.57	0.22	0.10	0.09	0.42	0.01	0.05
ASD1	22.56	1,924	42,691	22.1	174	118	24.1	16.0 b	25.05	0.21	0.22	0.11	0.54	0.02	0.07
ASD2	18.95	2,632	49,705	11.2	316	235	48.0	42.9 a	13.40	0.25	0.15	0.08	0.77	0.00	0.04
<i>P-value</i>	<i>0.12</i>	<i>0.07</i>	<i>0.38</i>	<i>0.09</i>	<i>0.30</i>	<i>0.23</i>	<i>0.08</i>	<i>0.01</i>	<i>0.11</i>	<i>0.98</i>	<i>0.07</i>	<i>0.51</i>	<i>0.22</i>	<i>0.32</i>	<i>0.08</i>

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680 ^z Reported values are averages of four replications. Means followed by different letters within each column and location are significantly different
681 at $\alpha = 0.05$ by Duncan's multiple range test. ^y CSF (chemical soil fumigation), ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses),
682 ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses).

683 **Table 6.** Soil treatment effects on the nutrient concentration of the soil solution extracted with suction lysimeters at 30 cm depth in Immokalee in
684 the spring of 2015. ^z

DATA ^y	Soil treatment ^x	NO ₃ ⁻		P		K		Ca		Mg		Na		Fe		B		Cu		Zn		Mn		Mo		Ni	
		mg L ⁻¹																									
7	CSF	134.0	a	20.4	b	30.1	c	189.0	de	43.0	ed	25.1	c	0.26	b	0.30	bc	0.51	a	2.93	cb	1.43	d	0.00	b	0.04	de
	ASD1	5.6	b	64.6	a	587.2	a	397.8	ab	100.2	a	91.1	a	15.06	a	0.49	abc	0.64	a	11.84	a	10.44	a	0.03	a	0.13	a
	ASD2	7.7	b	33.7	b	461.0	ab	431.4	a	88.2	ab	65.9	b	3.40	ab	0.68	abc	0.24	b	2.64	cb	7.75	ab	0.00	b	0.10	ab
14	CSF	160.1	a	28.4	b	41.2	c	225.4	cd	51.1	d	28.1	c	0.48	b	1.16	a	0.59	a	3.38	cb	1.88	cd	0.00	b	0.05	cd
	ASD1	4.1	b	90.9	a	401.9	ab	316.8	bc	67.2	bcd	57.2	b	11.81	ab	0.19	c	0.31	b	5.55	b	8.39	ab	0.00	b	0.09	abc
	ASD2	8.3	b	72.0	a	454.2	ab	383.0	ab	82.4	abc	73.4	ab	12.65	ab	0.45	abc	0.14	b	3.06	cb	8.33	ab	0.00	b	0.10	ab
21	CSF	20.1	b	27.1	b	22.8	c	106.4	e	22.8	e	16.9	c	0.11	b	1.02	ab	0.23	b	1.03	c	0.94	d	0.00	b	0.00	e
	ASD1	7.6	b	85.1	a	237.2	bc	188.0	de	46.4	ed	53.3	b	3.74	ab	0.18	c	0.15	b	2.15	cb	4.70	bcd	0.00	b	0.05	cd
	ASD2	5.5	b	89.7	a	356.5	ab	256.8	cd	61.4	cd	55.3	b	8.54	ab	0.23	bc	0.13	b	1.64	c	5.71	bc	0.00	b	0.06	bcd
<i>P value</i>																											
Soil treatment (A)		0.0001		0.0001		0.0001		0.0001		0.0001		0.0001		0.001		0.002		0.0001		0.0001		0.0001		0.06		0.0001	
DATA (B)		0.01		0.0001		0.0004		0.0001		0.0001		0.0004		0.30		0.65		0.0001		0.0001		0.002		0.06		0.0001	
A × B		0.002		0.01		0.01		0.19		0.05		0.002		0.11		0.004		0.0001		0.0001		0.11		0.03		0.34	

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686 ^z Reported values are averages of four replications. Means followed by different letters within each column and location are significantly different

687 at $\alpha = 0.05$ by Duncan's multiple range test. ^y DATA (days after treatment application). ^x CSF (chemical soil fumigation), ASD1 (anaerobic soil

688 disinfestation with 13.9 m³ ha⁻¹ of molasses), ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses).

Figure 1. Mean, maximum, and minimum soil temperature recorded at 5 and 10 cm depth, as affected by soil treatments in Immokalee and Citra during the spring of 2015. CSF (chemical soil fumigation, with Pic-Clor 60 at the rate of 224 kg ha⁻¹ in Immokalee, and Paladin™ at the rate of 496 L ha⁻¹ in Citra); ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter); ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter).

Figure 2. Volumetric soil water content, measured at 10 cm depth, as affected by soil treatments in Immokalee and Citra, in the spring of 2015. CSF (chemical soil fumigation, with Pic-Clor 60 at the rate of 224 kg ha⁻¹ in Immokalee, and Paladin™ at the rate of 496 L ha⁻¹ in Citra); ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter); ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter).

Figure 3. Mean hourly soil redox potential (Eh) as affected by soil treatments in Immokalee and Citra, in the spring of 2015. CSF (chemical soil fumigation, with Pic-Clor 60 at the rate of 224 kg ha⁻¹ in Immokalee, and Paladin™ at the rate of 496 L ha⁻¹ in Citra); ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter); ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter).

Figure 4. Soil treatment effects on N₂O flux ($\mu\text{g m}^{-2} \text{h}^{-1}$) measured on top of beds mulched with totally impermeable film (1, 2, 3 or 4 days after treatment application) and after punching the mulching film (21 days after treatment application) in Immokalee and Citra, in the spring of 2015. CSF (chemical soil fumigation, with Pic-Clor 60 at the rate of 224 kg ha⁻¹ in Immokalee, and Paladin™ at the rate of 496 L ha⁻¹ in Citra); ASD1 (anaerobic soil disinfestation with 13.9 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter); ASD2 (anaerobic soil disinfestation with 27.7 m³ ha⁻¹ of molasses, and 22 Mg ha⁻¹ of composted poultry litter). Reported values are averages of four replications. Means followed by different letters are significantly different at $\alpha = 0.05$ by Duncan's multiple range test.