Soil physicochemical (colloidal) properties affected by ozonated water and organic fertilization

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Abstract

More has to be investigated on the use of ozonated water (O₃) for the improvement of growth medium properties. Accordingly, the objective was to examine the effects of O₃ (control, 0.5, 1.0, and 2.0 mg L⁻¹) on soil physicochemical (colloidal) properties using organic fertilization (manure), under non-planted or planted conditions. Different soil physicochemical (colloidal) properties including soil available water (SAW), aggregate stability, soil porosity, pH, salinity (EC), organic carbon (SOC), CaCO₃, and cation exchange capacity (CEC) were determined. The experimental treatments and their interactions significantly ($P \leq 0.05$) affected soil physicochemical properties including SAW (4.17–10.98%), aggregate stability and porosity (7.77–57.37%), SOC (0.15–2.09%), and CEC (17.68–42.75 Cmol(+)/kg). Interestingly, the single use of O₃ or in combination with manure significantly decreased EC. Although O₃ significantly decreased SOC in non-planted soils, it significantly increased SOC in planted soils. O₃ may enhance soil physicochemical (colloidal) properties, and if combined with manure in a planted soil, such positive effects may be further enhanced.

Keywords Aggregate stability · Soil available water · Cation exchange capacity · Organic carbon · Ozonated water

1 Introduction

The healthy and sustainable production of agricultural crops is essential for feeding the world increasing population. Different methods and techniques have been so far used to increase agricultural and horticultural products [1, 2] including organic farming, biological fertilization, plant growth regulators, and ozonated water [38, 47]. The technique of ozonated water is among the newest ones and has been used for different purposes including the disinfection of waste and polluted waters. Ozone is produced in the atmosphere by the catalysis of ozone molecule into oxygen atoms, which then react with oxygen (O₂) molecules and produce ozone [4, 35, 37, 50].

Due to the high oxidizing potential of ozone molecules, they can significantly influence soil properties. For example, ozone can disinfect soil medium and mineralize organic matter available in different parts of the soil including soil colloids [8, 11]. Accordingly, ozone may enhance soil fertility

affecting plant growth and microbial activities. However, as an oxidizing molecule, ozone may significantly mineralize soil organic matter resulting in the degradation of soil structure. Accordingly, manure was also tested as an experimental treatment to test if it can alleviate the negative effects of ozone on soil properties.

Due to the non-stability of ozone molecule, it is produced at the place of consumption. Ozonated water in the present research was produced using an ozone instrument generator, which is able to turn O₂ molecule into oxygen atoms at high voltage, and in combination with O₂ produce O₃. The solubility of O₃ in water is not high, so using a diffuser its solubility was optimized [47]. One of the important factors, which determines ozone solubility, is water pH. Some ozone molecules are decomposed with increasing water pH (due to the presence of OH⁻), which is enhanced by organic compounds affecting ozone half-life. For example, in the typical drinking water, ozone half-life is between 1 and 20 min. However, carbonate ion can prevent the degradation of ozone in water (Hoigné, 1988).

Although there is not much research on the effects of ozonated water (O₃) on plant growth, a few experiments have so far indicated the positive effects of O₃ on plant growth under different conditions including stress. For example,

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Peykanpour et al. [39] investigated the effects of O₃ on cucumber (*Cucumis sativus* L.) growth under salt stress. They found that the O₃ treatment (0.5 and 1.0 mgL⁻¹) could improve cucumber growth under the stress by significantly increasing plant fresh and dry weights, fruit yield, and plant leaf area.

In another research, Graham et al. [16] investigated the effects of O₃ on the growth of tomato (*Lycopersicon esculentum* L.) and similarly found that ozone at 3.0 mgL⁻¹ significantly increased tomato fresh weight and leaf area, and significantly decreased algal growth. Accordingly, such researchers indicated that the proper concentration of ozone (oxygen) in water can improve plant growth by increasing plant metabolism. Peng et al. [38] examined the effects of diluted sludge treated with ozone, on lettuce growth (*Lactuca sativa* L.) and found although this treatment was not significant on lettuce growth and production, it significantly improved lettuce biochemical properties by increasing chlorophyll, ascorbic acid, and soluble sugar, and by decreasing plant nitrate.

Graham et al. [16] speculated that because of a strong oxidizing potential [19], O₃ can also affect plant growth, by increasing the availability of soil nutrients for plant and soil microbes. The other interesting effect of ozonation is on the disinfection of microbes including fungi, bacteria, and viruses [12, 36]. For example, even the potential use of ozone for controlling the pandemic Coronavirus has been suggested and tested [48]. The mineralizing effects of ozone on soil organic matter can be through the production of OH radicals resulted by the catalytic decomposition of ozone [11, 41]. However, organic fertilization (manure) can also alleviate the negative effects of ozone on soil physicochemical properties by acting as a substrate for ozone, and reducing the oxidization of soil organic matter by ozone.

Plant presence can also importantly affect soil physicochemical and microbial properties, mainly due to the effects of rhizosphere [42]. However, to our knowledge, there is not any data on the combined effects of O₃, plant presence, and manure affecting soil physicochemical properties. Tahamolkonan et al. [47] just indicated the use of O₃ and organic fertilization (manure) can improve tomato growth and fruit quality by affecting plant biochemical properties including total soluble solids, total sugar, chlorophyll contents, and vitamin C (ascorbic acid).

Although according to the abovementioned details, the direct effects of O₃ on plant growth and production have been researched, to our knowledge, there is little data on O₃ affecting soil physicochemical (colloidal) properties. Organic fertilization such as manure can also enhance soil physicochemical (colloidal) properties. However, the combined effects of manure and O₃, in a planted growth medium, have yet to be investigated. It has been proposed in this research that although one of the main reasons for

the positive effects of ozonated water on plant growth is the increased availability of oxygen for plant use, it may also improve plant growth by enhancing the growth medium (soil) properties.

With respect to the abovementioned details, one important question, which has been rarely investigated, and requires more investigation, is the effects of O₃ on soil physicochemical (colloidal) properties. It is also pertinent to provide an organic medium such as manure to determine if different soil properties are differently affected by O₃, due to the increased availability of nutrient in a non- or planted-soil.

2 Materials and methods

2.1 Experimental site

The experiment was conducted in the Research Greenhouse of Islamic Azad University (Isfahan Branch), Isfahan, Iran, from the November of 2014 to the June of 2015, at the eastern longitude and northern latitude of 51° and 37' and 35° and 47', respectively, 1549 m above the sea level. The area of the Research Greenhouse is equal to 270 m², covered with a poly ethylene layer with 4.5% of an ultraviolet (UV) micron layer, resistant to UV, with an arch-like structure and three halls, each 45 m big. The pots were irrigated with hand; the greenhouse has a heating (heater) and cooling (cooler) system (15–27 °C), a thermometer, humidity meter, and a gate on the ceiling to aerate the greenhouse (Fig. 1).

2.2 Ozonation

The following concentrations of ozonated water (O₃) were tested in this experiment: control (0), 0.5, 1, and 2 mg L⁻¹ produced using the following ozone generator (brand: Parto Parsezesh, Iran) [47]. The capacity of ozone generator is 70 mg ozone per minute, measuring 180 × 360 × 420 mm, and weighing 11 kg. The voltage is 220 V, with a fan, a resistant gas tube, a controlling system to avoid water entering the generator, a compressor to supply air for the generator, and a one-way ozone valve to avoid reentering ozone.

It is notable the concentrations are ozone concentration in water, which were used to dilute the nutrient's stock solution used to irrigate the plants. The oxygen (with the purity of 21%) was entered into the generator by an internal valve and it was circulated between the two electrodes, isolated by a dielectric. At high voltage, the electrodes catalyze the oxygen molecules into two oxygen atoms, which react with an oxygen molecule, resulting in the production of ozone molecules, directed out of the instrument using an external valve. Since ozone is not much soluble in water (14 mM. L⁻¹ at 20 °C), using a diffuser the process of ozone solubilization

Fig. 1 Different stages of the experiment including: **a** seedling growth, **b** transplantation of seedlings, **c** the process of ozonating water, **d** growth of tomato plants in the greenhouse, **e** organic fertilized soil with manure, **f** soil measurements, **g** tomato plants treated with control fertilization (without manure) and Oz3, and **h** tomato plants treated with organic fertilization (manure) and Oz3

was optimized; the diffuser was connected to the external valve by a tube. The diffuser was made of sand and silt, with the sides of $20 \times 20 \times 20$ mm, and ozone was diffused from bottom to top.

The final concentration of ozone in water was determined using ozone test kit. The medium used for the experiment consisted of a control soil and the combination of a control soil and organic medium (ship manure). The pots containing the experimental medium were non-planted (control) and planted.

The gas (ozone) flow rate by the instrument is 70 mg min^{-1} , and because the solubility of ozone in water (at 20°C) is 672 mg L^{-1} (14 mol), the gas flow rate at 0.5, 1, and 2 min, injected into 1L of tap water, resulted in O₃ with the ozone concentrations of 0.5, 1.0, and 2.0 mg L^{-1} . The stock solution containing the nutrients was immediately diluted with the prepared O₃ and was used to irrigate the pots. Due

to the process of sterilization and disinfection, tap water contains negligible amount of organic matter and dissolve inorganic. The plants were treated with the O₃ treatment for five months. The plants were irrigated with O₃ when the pot moisture was near the wilting point. Each pot contained 3 kg of soil and was irrigated with 2 L of O₃. The transplantation of seedlings was conducted at three-leaf growth stage. The seedlings were first irrigated with tap water, and after the establishment, they were irrigated with O₃. All the measured parameters were determined at harvest.

2.3 Experimental treatments

The experiment was a split-split plot in which the soil treatments were devoted to the main plots, planting to the sub-plots, and O₃ to the sub-sub plots. The experiment consisted of 16 treatments (with three replicates) including the pots containing control soil (S1) or control + organic fertilized soil (manure with 56% moisture, at 1.5 kg per plot) (S2), non-planted (P1) and planted (P2), and irrigated with O₃ at control (Oz1), 0.5 (Oz2), 1.0 (Oz3), and 2.0 (Oz4) mg L^{-1} . The plots measuring $80 \times 40 \times 40 \text{ cm}$ (L $\times$ W $\times$ H) were transplanted with tomato seedlings at the V3 (three-leaf growth) stage (27/10/2014). The chemical properties of manure were according to the following: pH: 7.8, EC: 18.6, OC: 15.9%, N: 2.26%, P: 1.23%, K: 2.3%, Ca: 2.1%. The control soil used for the experiment was a clay loam [14], with the pH of 7.6, salinity of $904 \mu\text{S cm}^{-1}$ [43], organic matter of 1.5% [32], and bulk density of 1.3 g cm^{-3} [28].

2.4 Measurements

Different soil physicochemical properties including the stability of soil aggregates, mean weight diameter (MWD), geometry mean diameter (GMD), soil texture, soil available water (SAW), salinity (EC) and acidity (pH) of a saturated paste, organic carbon (SOC), cation exchange capacity (CEC), CaCO_3 , and morphological properties of the experimental soil were determined.

2.4.1 Stability of soil aggregates

The stability of soil aggregates was measured using the wet sieving method [3]. The soil samples were first air-dried, sieved through a 4.75-mm mesh (#4), and moistened using a plastic tray. The soil aggregates were then meshed by the sizes of 2.0, 1.0, 0.5, 0.25, and 0.05 mm (30 rounds per minute) for 5 min. The meshes were then removed from the water, and the remaining aggregates on each mesh were washed and finally dried at 65°C . Due to the presence of sand, the stability of aggregates had to be adjusted. Accordingly, the aggregates were spread on the same sizes of mesh, oven dried, weighed, and deducted from the initial weight

of the aggregates on each mesh, and finally, the aggregates were precisely determined. The two parameters of MWD and GMD were also calculated using the following formulas.

$$MWD = \sum_{i=1}^n \bar{X}_i W_i$$

In which “ $\bar{X}_i$ ” is the mean weight diameter on each mesh with the size of “ i ” (the mean diameter of the meshes), n is the number of meshes, and W_i is the ratio of each aggregate weight to the total weight of the soil excluding the weight of sand.

GMD was calculated using the following formula:

$$GMD = \exp\left(\frac{\sum_{i=1}^n W_i \log \bar{X}_i}{\sum_{i=1}^n W_i}\right)$$

2.4.2 Soil available water

Soil available water (SAW), defined as the difference between the maximum amount of water (field capacity), held by soil and wilting point, which there is not any water available for plant use, was determined using the following method (Cassel, D.K. and Nielsen, D.R., 1986). The metal tubes containing the soil samples were inserted into a pressure-plate instrument, and the moisture of soil samples was measured at the suctions of 0.3 and 15 bars. Accordingly, to saturate the tubes, they were inserted into water for 24 h, and their moisture was determined using the instrument.

2.4.3 Salinity and acidity

Using 300 g of meshed soil, saturated with distilled water for 24 h, the samples were extracted with Buckner Funnel (Model B-55), and then, the salinity (EC) of soil samples was determined using a conductivity meter (Model 712) at 25 °C. The acidity of the saturated paste was also determined using a pH meter (Metrohm, model 827) (Klute, 1986).

2.4.4 SOC

Using the wet oxidation method (potassium bichromate and sulfuric acid), and by the reversible titration with ammonium ferrous sulfate, SOC was determined [49].

2.4.5 CEC

Using the sodium acetate method, soil CEC was determined according to the following stages: (1) saturation with sodium acetate, (2) rinsing the cations with ethanol 95%, (3) exchanging sodium (Na^+) by ammonium (NH_4^+) using sodium

acetate, and (4) measuring Na^+ using flame photometer, which was calculated on the basis of meq/100 g soil.

Accordingly, 2.5 g air-dried soil was poured into the centrifuge tubes, treated with 25 mL sodium acetate 1 N and shaken (2500 g) for 5 min, and the supernatant was poured away (repeated twice). The same method was also used by white ethanol, and at the third time, the EC of supernatant was measured to make sure its salinity is less than $40 \mu\text{S cm}^{-1}$; if it was higher, more rinsing was done. In the next stage, each sample was treated with 25 mL sodium acetate 1 N, just similar to the abovementioned details (repeated twice); however, this time the supernatant was collected and poured into a 50-ml volumetric flask, which was brought up to volume to measure Na^+ concentration using the flame photometer [44].

2.4.6 CaCO_3

Soil CaCO_3 was measured according to the following: 1 g air-dried soil was meshed, and poured into a 250-ml volumetric flask, and was treated and shaken well with 20 ml HCl 1 N. The samples were cool down and treated with 5 mL phenolphthalein indicator and NaOH 1 N until the appearance of purple color.

2.4.7 Micromorphology of soil samples (quantitative study of soil porosity)

Using the following method, the quantitative study of soil porosity in the thin slices of soil samples was conducted. The undisturbed soil samples were air-dried and were saturated using polyester resin containing 60% resin, 40% acetone (diluter), 14 drops of catalyst, and seven drops of cobalt. The samples were gradually saturated under vacuum with the addition of resin; it took 6 weeks, for hardening the samples [34]. The sections of the samples were observed using the polarizan microscope, and from each section, one picture was taken under normal polarizan, and was investigated using the related software to determine the soil porosity of < 2 , $2\text{--}10$, and $> 10 \mu\text{m}$ [13].

2.5 Statistical analyses

Data were subjected to analysis of variance using SAS. Means were compared by least significant difference (LSD) at $P \leq 0.05$.

3 Results

3.1 Analysis of variance

According to the analysis of variance, none of the experimental treatments significantly affected soil pH. However,

the single and the interaction effects of the experiential treatments were significant on soil EC. Interestingly, organic fertilization (manure) did not affect SOC, which was significantly affected by the other experimental treatments including plantation and O3 and their interaction. The other soil properties including CaCO_3 , SAW, and CEC were also significantly affected by the single and interaction effects of the experimental treatments (Table 1).

3.2 SAW

O3 significantly increased SAW, and with increasing ozone concentration, such effects became clearer as at the highest level of ozone concentration, under different experimental treatments, the highest SAW was resulted. In the control soil, there was a significant difference between plantation and non-plantation treatments, at different ozone concentration (excluding Oz4). However, when combining organic fertilization (manure) with the control soil, the effects of plantation became apparent as SAW significantly increased in the planted soil, related to the non-planted soil. The least SAW (4.17%) was resulted by S2P2Oz1 (fertilized soil, planted and not ozonated), and the highest ones (10.98 and 10.13%) were related to S1P2Oz4 (fertilized soil, not planted and ozonated at 2 mg L⁻¹) and S1P1Oz3 (control soil, not planted and ozonated at 1.5 mg L⁻¹), respectively (Table 2).

3.3 pH

There were not any significant effects of the experimental treatments on soil pH ranging from 8.04 (S2P2Oz4, fertilized soil, planted and ozonated at 2 mg L⁻¹) to 8.31 (S1P2Oz4, control soil, planted and ozonated at 2 mg L⁻¹) (Table 2).

3.4 Salinity

The single and the interaction effects of the experimental treatments significantly affected soil salinity. Interestingly, in most cases, O3 significantly decreased soil salinity compared with control. For example, in the control and planted soil, treatments Oz3 (0.59 dS m⁻¹) and Oz4 (0.63 dS m⁻¹) significantly decreased soil salinity, compared with control (0.93 dS m⁻¹). Similarly, in the organic fertilized planted soil, Oz2 (1.05 dS m⁻¹), Oz3 (0.87 dS m⁻¹), and Oz4 (0.72 dS m⁻¹) significantly reduced soil salinity compared with control (1.33 dS m⁻¹). The least and the highest soil salinity levels (0.59 and 1.93 dS m⁻¹) were resulted by treatments S1P2Oz3 (control soil, planted and ozonated at 1 mg L⁻¹) and S2P1Oz2 (fertilized soil, not planted and ozonated at 0.5 mg L⁻¹). However, just in the case of organic, non-planted soil, O3 increased soil salinity from 0.60 dS m⁻¹ in control (Oz1) to 1.93 (Oz2), 1.37 (Oz3), and 1.04 dS m⁻¹ (Oz4) (Table 2).

3.5 Organic carbon

In control and organic fertilized non-planted soils, O3 significantly decreased SOC compared with non-ozonated treatment. However, the presence of plant in the soil significantly increased SOC as treatments S1P1Oz4 (control soil, not planted and ozonated at 2 mg L⁻¹) and S2P2Oz4 (fertilized soil, planted and ozonated at 2 mg L⁻¹) resulted in the least (0.51%) and the highest (2.09%) SOC, respectively (Table 2).

Table 1 Analysis of variance indicating the effects of experimental treatments on different soil physicochemical properties

Sources of variation	d.f	SAW	pH	Salinity	SOC	CaCO_3	CEC
B	2	0.83 ns	0.012 ns	0.004 ns	0.02 ns	26.44 ns	4.78 ns
O.F	1	62.43*	0.058 ns	1.45**	0.08 ns	198.86*	46.77*
Error A (B. × O.F.)	2	0.64	0.018	0.01	0.03	2.73	0.57
P	1	7.84*	0.001 ns	0.03**	0.01 ns	218.03**	240.31**
O.F. × P	1	2.20 ns	0.00005 ns	0.09*	3.48**	531.34**	268.29**
Error B (B. × P. × O.F.)	4	0.41	0.010	0.009	0.01	6.82	3.88
Ozone	3	22.44**	0.005 ns	0.25**	0.70**	25.00**	330.82**
O.F. × ozone	3	0.26 ns	0.006 ns	0.23**	0.55**	37.12**	125.41**
P × ozone	3	1.54	0.010 ns	0.49**	1.33**	137.65**	190.07**
O.F. × P. × ozone	3	2.75	0.003 ns	0.29**	0.44**	99.14**	5.06 ns
Error	24	0.22	0.008	0.01	0.01	2.58	2.98
C.V. (%)		6.26	1.10	10.72	10.69	11.01	6.78

d.f. degree of freedom, SOC soil organic carbon, CEC cation exchange capacity, SAW soil available water, B. block, O.F. organic fertilization, P. planting, ns not significant, * and ** significant at $P=0.05$ and 0.01, respectively

Table 2 The interaction effect of experimental treatments including ozonated water (O3), organic fertilization, and planting on different soil physicochemical properties

Organic fertilization	Planting	Ozone (mg L ⁻¹)	SAW (%)	pH	Salinity (dS m ⁻¹)	SOC (%)	CaCO ₃ (%)	CEC (Cmol(+)/kg)
Control soil	Not planted	Control	7.81 ± 1.75 cd	8.22 ± 0.06	0.81 ± 0.05def	1.09ef	15.00c	22.90 ± 1.34 fg
		0.5	9.39 ± 0.33b	8.16 ± 0.13	0.74 ± 0.13fgh	0.54 g	25.83a	30.72 ± 0.95 cd
		1	10.13 ± 0.13ab	8.16 ± 0.13	0.96 ± 0.07 cd	0.53 g	20.00b	17.6 ± 1.148 h
		2	10.01 ± 3.22b	8.18 ± 0.06	0.70 ± 0.04fgh	0.51 g	27.50a	26.96 ± 1.64e
	Planted	Control	6.70 ± 0.26fgh	8.18 ± 0.06	0.93 ± 0.1cde	1.11ef	20.00b	19.71 ± 2.04 h
		0.5	7.02 ± 0.16dg	8.18 ± 0.06	0.77 ± 0.03efg	1.06ef	12.17cde	20.43 ± 0.79gh
		1	7.70 ± 0.45cde	8.20 ± 0.06	0.59 ± 0.07 h	1.43c	6.25f	24.06 ± 2.50f
		2	10.98 ± 1.87a	8.21 ± 0.1	0.63 ± 0.09gh	1.34 cd	6.25f	33.04 ± 1.64c
	Soil + organic fertilization	Control	5.28 ± 0.42i	8.19 ± 0.09	0.74 ± 0.29 h	1.71b	11.67de	19.13 ± 2.09 h
		0.5	6.18 ± 0.17gh	8.09 ± 0.11	1.93 ± 0.35a	1.03f	12.50cde	18.99 ± 1.39 h
		1	6.84 ± 0.33efg	8.08 ± 0.16	1.37 ± 0.15b	1.21de	15.00c	20.29 ± 1.84gh
		2	8.21 ± 0.44c	8.09 ± 0.01	1.04 ± 0.20c	1.18def	6.27f	28.84 ± 1.84de
	Planted	Control	4.17 ± 0.89j	8.12 ± 0.09	1.33 ± 0.31b	0.25 h	18.33b	22.61 ± 1.37 fg
		0.5	5.94 ± 0.57hi	8.12 ± 0.10	1.05 ± 0.15c	0.15 h	10.83e	20.29 ± 1.60gh
		1	7.13 ± 0.23def	8.20 ± 0.03	0.87 ± 0.14def	0.62 g	11.67de	38.41 ± 2.50b
		2	7.75 ± 0.41 cd	8.26 ± 0.06	0.72 ± 0.03fgh	2.09a	14.17 cd	42.75a
	LSD			0.16	0.17	0.19	2.96	2.86

SAW soil available water, SOC soil organic carbon, CEC cation exchange capacity. Means followed by the same letter are not significantly different at $P=0.05$ using least significant difference (LSD) test

3.6 CaCO₃

O3 significantly increased CaCO₃ in non-planted soil; however, in planted soils, CaCO₃ significantly decreased with increasing ozone concentration. The least (6.25%) and the highest percentages (27.50%) of CaCO₃ were resulted by treatments S1P2Oz4 (control soil, not planted and ozonated at 2 mg L⁻¹) and S1P1Oz4 (control soil, not planted and ozonated at 2 mg L⁻¹), respectively (Table 2).

3.7 Cation exchange capacity

According to the results, O3, especially Oz3 and Oz4, significantly increased cation exchange capacity (CEC) under different treatments. Planting, especially in the organic fertilized soil, significantly increased CEC. The least (17.68 cmol(+)/kg) and the highest (42.75 cmol(+)/kg) CEC were resulted by S1P1Oz3 (control soil, not planted and ozonated at 1 mg L⁻¹) and S2P2Oz4 (fertilized soil, planted and ozonated at 2 mg L⁻¹), respectively (Table 2).

3.8 Soil aggregates

Analysis of variance indicated the significant effects of planting, O3, and their interaction with organic fertilized soil on MWD. However, GWD was significantly affected by all the experimental treatments and their interactions. With

increasing the size of soil porosity, the significant effects of the experimental treatments and their interactions became clearer as at > 10 µm, all the experimental treatments and some of their interactions significantly affected soil porosity with maximum values in the range of 10.1–13.1% (Table 3).

3.9 MWD and GMD

The results indicated that O3 significantly increased MWD in P1 treatments as treatments S1P1Oz2 (control soil, not planted and ozonated at 0.5 mg L⁻¹) and S2P1Oz3 (fertilized soil, not planted and ozonated at 1 mg L⁻¹) resulted in the highest (0.847 and 0.870 mm) MWD values. However, the least MWD values (0.593 and 0.540 mm) were related to the P2 treatments by S1P2Oz4 (control soil, planted and ozonated at 2 mg L⁻¹) and S2P2Oz3 (fertilized soil, planted and ozonated at 1 mg L⁻¹). Similarly, the GMD values were also the highest under P1 treatments, and S1P1Oz2 (control soil, not planted and ozonated at 0.5 mg L⁻¹) and S2P1Oz3 (fertilized soil, not planted and ozonated at 1 mg L⁻¹) treatments had the highest (0.820 and 0.797 mm) GMD values (Table 4).

According to the results, O3 significantly increased soil porosity of 2 µm as treatment S1P1Oz4 (control soil, not planted and ozonated at 2 mg L⁻¹) resulted in the highest value (57.37%), significantly higher than the control treatment (48.43%). There was not a clear trend

Table 3 Analysis of variance indicating the effects of organic fertilization, ozonated water, and their interaction on MWD, GMD, and soil porosity in each aggregate size (μm) including <2, 2–10, and >10

		Mean of squares				
Sources of variation	d.f	MWD (mm)	GMD (mm)	<2 (mm)	2–10(μm)	>10 (μm)
B	2	0.003	0.0004**	70.60**	0.58	0.62*
O.F	1	0.008	0.002**	2.00	57.64	12.00**
Error a (B. $\times$ O.F.)	2	0.0006	0.000002	0.50	6.28	0.008
P	1	0.314**	0.070**	2.00	23.80**	21.07**
O.F. $\times$ P	1	0.003*	0.000002	2.00	7.36*	0.70*
Error b (B. $\times$ P. $\times$ O.F.)	4	0.0003	0.0002	0.50	0.74	0.04
Ozone	3	0.007**	0.003**	322.88**	3.03	17.73**
O.F. $\times$ ozone	3	0.016**	0.003**	2.00	4.48*	1.36*
P. $\times$ ozone	3	0.012**	0.0007	2.00	3.19	0.44
O.F. $\times$ P. $\times$ ozone	3	0.021**	0.006**	2.00	2.28	1.15
Error	24	0.001	0.0002	24	1.46	0.33
C.V		4.93	2.15	2.79	3.71	5.73

d.f. degree of freedom, MWD mean weight diameter, GMD geometric mean diameter, B. block, O.F. organic fertilization, P. planting, *, **: significant at $P=0.05$ and 0.01 , respectively

of soil, planting, and O₃ on the soil porosity of 2–10 μm . However, in the soil porosity of > 10 μm with increasing ozone concentration, soil porosity significantly increased. For example, in the treatments S1P1Oz, the highest value (10.10%) of > 10 μm was related to treatment S1P1Oz4 (control soil, not planted and ozonated at 2 mg L⁻¹) significantly higher than the control treatment (8.43%); in the treatments S2P2zO, the highest value (13.13%) of > 10 μm was resulted by S2P2Oz4 (fertilized soil, planted and

ozonated at 2 mg L⁻¹) significantly different from control (9.43%) (Table 4).

4 Discussion

It is important to use modern techniques, which may contribute to the health of the society and increase the production of food for the world increasing population. The improvement

Table 4 The interaction effect of experimental treatments including organic fertilization, planting, and ozonated water on MWD, GMD, and soil porosity (%) in each aggregate size (μm) including <2, 2–10, and > 10

Organic fertilization	Planting	Ozone (mg/L)	MWD (mm)	GMD (mm)	<2 (μm)	2–10 (μm)	>10 (μm)
Control soil	Not planted	Control	0.757 $\pm$ 0.01b	0.787 $\pm$ 0.01bc	48.43c	35.23ab	8.43hi
		0.5	0.847 $\pm$ 0.01a	0.820 $\pm$ 0.01a	44.73d	34.07abc	7.77i
		1	0.730 $\pm$ 0.05b	0.730 $\pm$ 0.02e	53.10b	33.37bcd	8.80gh
		2	0.753 $\pm$ 0.05b	0.750 $\pm$ 0.02de	57.37a	33.33b-e	10.10ef
		Control	0.573 $\pm$ 0.05de	0.690 $\pm$ 0.03 fg	N.A	33.53a-d	10.10ef
	Planted	0.5	0.610 $\pm$ 0.01d	0.690 $\pm$ 0.03 fg	N.A	35.57a	8.90gh
		1	0.600 $\pm$ 0.01d	0.703 $\pm$ 0.01f	N.A	32.93cde	11.20bc
		2	0.593 $\pm$ 0.02de	0.700 $\pm$ 0.02f	N.A	31.47def	11.17bcd
		Control	0.737 $\pm$ 0.05b	0.747 $\pm$ 0.01de	N.A	30.40f	9.43 fg
		0.5	0.570 $\pm$ 0.03de	0.673 $\pm$ 0.01gh	N.A	30.03f	10.30def
Soil + organic fertilization	Not planted	Control	0.730 $\pm$ 0.01b	0.767 $\pm$ 0.01 cd	N.A	32.63cde	9.43 fg
		0.5	0.717 $\pm$ 0.06bc	0.730 $\pm$ 0.02e	N.A	32.60cde	8.33hi
		1	0.870 $\pm$ 0.07a	0.797 $\pm$ 0.03ab	N.A	31.87def	10.53cde
		2	0.673 $\pm$ 0.01c	0.747 $\pm$ 0.01de	N.A	33.27b-e	11.77b
		Control	0.737 $\pm$ 0.05b	0.747 $\pm$ 0.01de	N.A	30.40f	9.43 fg
	Planted	0.5	0.570 $\pm$ 0.03de	0.673 $\pm$ 0.01gh	N.A	30.03f	10.30def
		1	0.540 $\pm$ 0.02e	0.657 $\pm$ 0.01 h	N.A	31.20ef	11.53b
		2	0.560 $\pm$ 0.03de	0.657 $\pm$ 0.01 h	N.A	29.97f	13.13a
		LSD	0.052	0.025	2.17	2.16	0.87

MWD mean weight diameter, GMD geometric mean diameter. Means followed by the same letter are not significantly different at $P=0.05$ using least significant difference (LSD) test. N.A. not available

of growth media for the production of agricultural and horticultural crops is an important aspect in this respect. In the present research, the effects of O₃ in a mineral soil singly or treated with organic fertilization (manure) non-planted and planted on soil physicochemical properties were investigated. Interestingly, the results indicated O₃ can improve the physicochemical (colloidal) properties of the growth medium, specifically a mineral soil. However, manure and plantation were also able to further enhance such properties. Such new findings and contributions can be useful for the preparation of growth media, which can significantly increase the growth of horticultural crops in controlled conditions. It is a safe and promising method, which can make a big difference in the properties of the growth medium, with affordable expenses, and is recommendable from health and environmental perspectives.

4.1 Analysis of variance

The results indicated that the experimental treatments were non-significant on soil pH meaning that other factors rather than O₃, manure, plantation affect soil pH or a longer time may be required. However, soil salinity was significantly decreased by the experimental treatments and their interactions indicating that the solubility of ions such as sodium (Na⁺), chloride (Cl⁻), calcium (Ca²⁺), and magnesium (Mg²⁺) is affected by O₃ and manure. Additionally, because soil CEC was significantly increased by the experimental treatments, the higher absorption of salt ions on the soil colloids can decrease their solubility in the soil solution and hence decreases soil EC. This can also be due to the higher availability of SOC by O₃, which was also intensified in the presence of manure and plant roots. According to the results under non-planting conditions, just the Oz3 and Oz4 treatments significantly decreased soil salinity; however, in the presence of plant in the soil, the Oz2 treatment was also able to significantly decrease soil salinity [30]. The higher production of CaCO₃ by O₃ can be due to higher production of CO₂, respiration of plant roots, and manure mineralization, which in combination with CaO can produce CaCO₃.

4.2 SAW

The experimental treatments significantly increased SAW. The higher mineralization of soil organic matter by ozone, and higher root growth (rhizosphere activities) in the presence of manure are the reasons for the improved structure of the soil and hence increased SAW. Treating soil with ozone improves the incorporation of organic matter, produced by the plant roots (organic acids), into the structure of the soil, and hence increases SAW. Higher micropores in organic matter, compared with mineral soil, hold higher amounts of water for plant use [9, 10].

4.3 pH

The pH of the soil is a characteristic, which under some conditions, such as the present research, can be subject to change in a longer period. This is interesting because an oxidizing molecule such as ozone can alter pH; however, the important reason for the non-significant changes of soil pH in the presented experiment can be due to soil buffering capacity, which increases in the presence of plant and manure. This can be a favorable characteristic of ozone and manure, for plant growth, because pH fluctuations may not positively affect plant growth [33, 45].

4.4 Salinity

The main reason for the reduction of soil salinity by ozone, plant, and manure can be the increased amount of organic matter. Previous research has indicated one of the methods, which efficiently decreases soil salinity, is the use of organic fertilization. Accordingly, Khaleda et al. [22] illustrated the molecular mechanisms by humic acid, which may decrease the negative effects of salinity on the growth of *Arabidopsis thaliana* seedlings. They suggested the possible roles of a high-affinity K⁺ transporter (HKT1), which can regulate the movement of sodium in plant. Humic acid was able to alleviate the negative effects of salt stress on root growth by the following: (1) regulating the activity of HKT1, (2) blocking the degradation of HKT1, (3) the enhanced distribution of Na⁺ to the elongation zone, and (4) the reabsorption of Na⁺ by the cells of xylem and parenchyma. Accordingly, this can also be the reason for the increased soil salinity, when plant is not present. It is because the salt ions are not absorbed by plant, and as a result, their concentration increases in the soil solution. Peykanpour et al. [39] found O₃ can reduce the unfavorable effects of salinity on the growth of cucumber. O₃, as an oxidizing molecule, can increase the mineralization of soil organic matter, and increase the availability of functional groups, which absorb higher salt ions from the soil solution and decrease soil salinity.

4.5 SOC

The important effect of O₃ on SOC is through the oxidation process [29, 41] affecting the properties of soil colloids and the subsequent absorption or release of the elements by the soil colloids (the significant increase of soil CEC by the experimental treatments). According to our results, soil physical properties including SAW, aggregates, and porosity are also affected by O₃, manure, and plantation, affecting the absorption, release, and movement of elements in the soil [7]. Manure can improve the colloidal properties of the soil, because it acts as an organic phase [45]. Ozone not

only increases the rate of chemical oxidation in the soil, by enhancing oxygen (O_2) concentration [51], it can also accelerate the rate of biochemical oxidation by increasing the respiration of plants and soil organisms.

The results indicated that O_3 decreased SOC, which is due to the higher O_2 in the soil, significantly increasing the chemical and biochemical oxidation of SOC [24]. However, plant presence increased SOC, which is mainly due to the biological activities of plant roots and their production of different organic products, increasing the solubility of nutrients and the production of microbial biomass.

4.6 Plant presence

Plant presence in the soil can significantly affect soil physicochemical and biological properties [7, 17, 18]. However, in the case of irrigating plant with O_3 , due to higher O_2 concentration, plant presence may affect soil physicochemical and biological properties according to the following. Higher O_2 concentration increases plant growth including plant roots, which can (1) bind the soil particles and enhance soil structure, (2) release different products including organic acids and increase the solubility of different nutrients for plant and microbial use, and (3) increase soil biological activities including their interactions with the host plants, enhancing nutrient availability, biological fixation of nutrients, controlling pathogens, and alleviating stress [26].

4.7 $CaCO_3$

Although the effects of the experimental treatments were not significant on soil pH, their significant effects on soil $CaCO_3$ indicate that if the experimental treatments are tested in a longer time and a large area, it would be possible to adjust soil pH to the favorite value. Increasing soil $CaCO_3$ by the experimental treatments can be due to the enhanced colloidal properties of the soil such as the availability of SOC and nutrients and the improved structure of the soil such as the stability of aggregates. Increasing ozone concentration increased $CaCO_3$, which can be due to a more favorite soil medium by affecting soil physical, chemical, and biological properties [21, 31].

Increasing $CaCO_3$ in non-planted soil by O_3 can be due to the increase of O_2 concentration by O_3 , which may provide more oxygen for the biological activities of soil microbes. Accordingly, more CO_2 is released, which react with CaO , resulting in the subsequent formation of $CaCO_3$. However, when planted, soil $CaCO_3$ decreased, which can be due to the production of acidic compounds by plant roots, catalyzing $CaCO_3$ to CaO and CO_2 , subsequently emitted from the soil [31].

4.8 MWD and GWD

The experimental treatments significantly increased the properties of soil aggregates including MWD and GWD as well as soil porosity. Such effects can be due to improving colloidal properties, by the increased amount of soil organic matter and CEC, affecting the binding of soil particles and the formation of soil aggregates [20]. The size of soil porosity was a determining factor on the experimental parameters, as for example, in the size of $> 10 \mu m$, they were more significantly affected by the experimental treatments.

The results by Zhu et al. [53] indicated that organic fertilization significantly increased the stability of soil aggregates by increasing MWD and GWD. Similarly, Cao et al. [5] determined how carbon cycle may be affected by different parameters including SOC and the stability of soil aggregates. They found that SOC significantly increased the stability indexes of soil aggregates, which collectively increased the aboveground biomass. Accordingly, precipitation was the most important factor, significantly affecting SOC and the stability of soil aggregates. Zhang et al. [52] found that the use of biochar as a source of organic fertilization can enhance soil properties in a 6-year rotation of rice–wheat cropping. Accordingly, among the other effects of biochar on yield production, the increased sequestration of carbon [46], which subsequently increased the stability of the soil aggregates, also considerably increased rice and wheat yield.

The use of O_3 significantly improved soil physical properties including SAW, and soil aggregate stability and porosity. Interestingly, the single use of O_3 or in combination with manure, significantly decreased soil salinity. The experimental treatments also significantly increased the colloidal properties of the soil such as SOC and CEC. Accordingly, the improvement of soil physicochemical properties by O_3 may significantly enhance the production of horticultural crops.

Another important aspect of irrigating plant with O_3 , investigated in our just published research [47], is the alteration of plant antioxidant activities. It can interestingly improve plant growth and fruit production under different circumstances including stress conditions and post-harvest during the storage, which is due to the activation of antioxidant pathways [25]. For example, Goffi et al. [15] found ozone can improve the antioxidant activity of ‘Soreli’ kiwifruit and increase its storability. It is because one of the important reasons for fruit senescence is the production of reactive oxygen species and oxidative stress.

5 Conclusion and future directions

The use of modern techniques, including ozonated water (O_3), may be a promising approach for the higher and safer production of agricultural and horticultural crops. However,

the subsequences of such a method on the growth medium properties demand more investigation. In this research, the physicochemical (colloidal) properties of the soil affected by O₃, manure, and plantation presence were examined. The results indicated that the single and the combined use of the experimental treatments significantly enhanced soil physical properties, including soil available water, aggregate stability and soil porosity, and chemical properties including salinity, organic carbon, CaCO₃, and cation exchange capacity. Interestingly, decreased soil salinity, increased organic carbon (in planted soil), and increased cation exchange capacity are among the most important effects of the experimental treatments on soil chemical (colloidal) properties. Although O₃ by itself proved to be an effective treatment significantly enhancing soil physicochemical properties, its combination with manure and plant presence intensified O₃ positive effects. It is safe and economic. It is possible to improve plant growth and yield production in a growth medium using the treatments tested in this research; however, more research is required to investigate plant growth under other circumstances including stress conditions. The tested method is a safe and economic method, and can be used for the treatment of irrigation water for increasing the yield of agricultural and horticultural crops. Future research may also investigate strategies, which may increase the efficiency of water ozonation for the improvement of growth medium properties with environmental and economic benefits.

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Declarations

Competing interests The authors declare no competing interests.

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